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The Elisha Mitchell Scientific Society

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JOURNAL
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Elisha Mitchell Scientific Society

Volume 75 November 1959 No. 2

PROCEEDINGS OF THE FIFTY-SIXTH ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE

The fifty-sixth annual meeting of the North Carolina Academy of Science was held at Catawba College, Salisbury, on May 1 and 2, 1959.

The Academy met in its first session at 10:30 a.m. on May 1 with President MacCarthy presiding. A general session with an invited paper was held on this occasion.

The business meeting of the Academy was held at 4:30 p.m. on May 1, at which time the following business was transacted:

The minutes of the 1958 meeting were approved as published in the Journal of the Elisha Mitchell Scientific Society **74:** 69–93.

Report of the Executive Committee

The Executive Committee met at the Geology Building in Chapel Hill at 7:45 p.m. on April 27, 1959. At this meeting the following matters were considered:

1. All persons who had applied for membership to this date were declared elected. Names of new members appear later in the Proceedings.
2. Two persons were elected to Life Membership in the Academy. These are S. G. Lehman, North Carolina State College, joined in 1920 and retired in 1956; and W. E. Speas, Wake Forest College, joined in 1921 and retired in 1959. Dr. Speas was President of the Academy in 1938.
3. The Committee voted to accept the invitation of the Woman's College of the U.N.C. in Greensboro to hold the 1960 meetings of the Academy on its campus.
4. John A. Yarbrough was named to represent the Academy at the AAAS in Chicago, December, 1959, and also at the Academy Conference at the same time. The incoming President and Secretary-Treasurer were empowered to name another member of the Academy to act as a second representative to the Academy conference.
5. The Committee voted to set up a committee of two members (to be named later) along with the Secretary-Treasurer to solicit new patron and sustaining members during the coming Academic year.
6. The Committee voted to increase the appropriation to the Elisha Mitchell Journal to $1 per member for the 1959 payment. It was formerly $0.50 per member. This is in addition to the $300 also given annually to the Mitchell Journal. The Secretary was instructed to use any remainder in the Research Fund to pay the 1959 increase in payments to the Mitchell Journal. No distinct policy beyond 1959 was set by the Committee.
7. The Secretary-Treasurer was instructed to prepare a summary of financial reports covering approximately a ten year period so that the Committee and the Academy could see any trends that may exist in our fiscal policies. This was suggested in lieu of preparing a formal budget for the Academy at this time.
8. Honorary membership for one year was voted to four outstanding physics teachers in North Carolina high schools selected by the committee of the Physics Section of the Academy. These persons are: Miss Estell McClees of Grainger High School, Kinston; John N. Scott, East Mecklenburg High School, Monroe; James N. Smith, Jr., New Bern High School, New Bern; and Robert B. Westbrook, Statesville High School, Statesville.
9. Junior membership for one year was voted to ten high school students who won prizes in the State Science Fair in 1959, and to seventeen students who placed high in the State Science Talent search for 1959. These names follow:

   Martin Violette, Claremont Central High School, Hickory; George C. Jackson, Jr., Winterville High School; Betty Johnson, Needham Broughton High School, Raleigh; James R. McCurry, Enka High School; David

E. Radford, Chapel Hill High School; Nancy M. Lawson, Oxford High School; Charles Funk, West Mecklenburg High School, Charlotte; Frank Starmer, Greensboro High School; Jackie Nolen, Farmville High School; Michael George, Greensboro High School. All the above were winners in the 1959 State Science Fair. Winners and honorable mention in the 1959 State Science Talent Search follow:

Michael D. Feezor, Salisbury; Robert D. Miller, Chapel Hill; Andrew D. Rogers, Jr., Raleigh; David W. Swain, Raleigh; William O. Wallace, Winston-Salem; Wendell J. Bouknight, Wilmington; William E. Byrd, Charlotte; Dillard L. DeHart, Asheville; Michael J. George, Greensboro; Paul C. Hardy, Jr., Charlotte; John W. Hollifield, Lenoir; William T. Jackson, Elizabeth City; Tommy G. Sharpe, Statesville; Charles F. Starner, Jr., Greensboro; Nancy F. Stovall, Wilmington; Clarence E. Styron, Morehead City; and Roger K. Teague, Hickory.

10. In a meeting in October the Executive Committee approved Academy sponsorship in the Second Junior Science Symposium. The other sponsor was the Office of Ordnance Research, U.S. Army on the campus of Duke University. A very successful Symposium was held on March 11, 12, and 13.
11. On January 30, 1959, a meeting of the Executive Committee approved the submission of a project on science education on the part of the Academy to the National Science Foundation for a total sum of $19,961.70. A committee consisting of Herbert E. Speece, Henry A. Shannon, B. B. Brandt, and J. A. Yarbrough had previously been appointed by the President to work up the full text of the project. The project was approved in full by the N.S.F. The Academy's project will involve seven short-term institutes for teachers of science in the high schools. Herbert E. Speece will direct the project and N. C. State College will act as fiscal agent. The project will operate during the next school term, 1959–60.
12. The following were elected to membership in the Academy or reinstated to active membership:

Ashworth, Ralph P., University of N. C., Chapel Hill
Battle, Ben H., Chapel Hill
Behre, Dr. Ellinor H., Black Mountain
Bell, Henry, 3rd, Beltsville, Md.
Bowers, Dr. Wayne A., University of N. C., Chapel Hill
Brett, Charles Everett, University of N. C., Chapel Hill
Brown, Dr. Henry S., N. C. State College, Raleigh
Burk, Carl John, University of N. C., Chapel Hill
Clarke, Dr. James W., Duke University, Durham
Councill, Richard J., Atlantic Coast Line R.R., Wilmington
DeWitt, Dr. Bryce S., University of N. C., Chapel Hill
DeWitt, Dr. Cecile, University of N. C., Chapel Hill
Dodson, Albert Carter, Chapel Hill
Doggett, Dr. Wesley Osborne, N. C. State College, Raleigh
Dumas, Ernest Mills, N. C. State College, Raleigh
Eads, James H., Jr., Meredith College, Raleigh
Festa, Salvatore A., Burlington City Schools, Burlington
Furr, A. K., Duke University, Durham
Lofton, Leroy Garner, University of N. C., Chapel Hill
Gold, Harvey S., Duke University, Durham
Harton, Frank N., Central High School, Charlotte
Herr, Dr. John Mervin, Jr., Pfeiffer College, Misenheimer
Holland, Miss Margaret M., University of N. C., Chapel Hill
Hopkins, Dr. John I., Davidson College, Davidson
Howell, Harry C., Jr., Central Scientific Co., Charlotte
Jackson, Dr. R. Bruce, Jr., Davidson College, Davidson
Johnson, J. Stanley, Senior High School, Greensboro
Jorgensen, Dr. Wilhelm, Office of Ordnance Research, Durham
Kaye, Stephen V., N. C. State College, Raleigh
Kim, Yoon Tuk, University of N. C., Chapel Hill
Kirk, Dr. Daniel E., Catawba College, Salisbury
Lane, Joseph R., Office of Ordnance Research, Durham
Lehman, Dr. Lillian Youngs, University of N. C., Chapel Hill
Martin, Mrs. Lorelle Franck, Mount Olive Junior High School, Mount Olive
McElrath, Mr. John P., N. C. State Highway Commission, Black Mountain

McGavock, Dr. W. G., Davidson College, Davidson
Morehead, Marcus B., University of N. C., Chapel Hill
Mohammad, Murad-Baba Murad, Duke University, Durham
Nelson, Dr. Richard R., N. C. State College, Raleigh
Newell, E. B., Catawba College, Salisbury
Nielsen, Dr. Walter M., Duke University, Durham
Powell, Robert W., Jr., Duke University, Durham
Ramseur, Dr. George S., University of the South, Sewanee, Tenn.
Ray, James Allen, N. C. State College, Raleigh
Routh, William, P. Lorillard Tobacco Co., Greensboro
Royal, Dr. Gladys, A. & T. College, Greensboro
Schmidt, Dr. Th.W., Office of Ordnance Research, Durham
Sowell, Jesse Clarence, Elon College, Elon College
Sowell, Mrs. Katye O., Elon College, Elon College
Straley, Dr. J. W., University of N. C., Chapel Hill
Todd, Daniel E., Jr., New Hanover High School, Wilmington
Ward, Lochlin M., Cardinal Products, Inc., Durham
Wiggs, Dr. Deems N., Atlantic Christian College, Wilson
Woods, Claiborne S., Jr., Cardinal Products, Inc., Durham
Yow, Dr. F. W., Western Carolina College, Cullowhee
Zebrowski, George, Wilmington College, Wilmington

Patron Members as of July 7, 1959

Governor Luther H. Hodges, Raleigh
W. Y. Preyer, Greensboro
C. M. Goethe, Sacramento, California
Richard E. Thigpen, Charlotte
Harold D. Cooley, House of Representatives, Washington, D. C.
Justice Emery B. Denny, Raleigh
Reuben B. Robertson, Asheville
C. P. Street, Charlotte
Irving E. Carlyle, Winston-Salem
Edward R. Zane, Greensboro
Burnham S. Colburn, Asheville
Thomas Pearson, Asheville

Sustaining Members as of July 7, 1959

Block-Southland Sportswear, Inc., Wilmington
E. I. du Pont de Nemours & Company, Kinston
Roxboro Cotton Mills, Roxboro
The Dickson Foundation, Inc., Mount Holly
Liggett & Myers Tobacco Company, Durham
Celanese Corporation of America, Charlotte
Acme Spinning Company, Belmont

The above reports and actions of the Committee were approved by the Academy in its business session on May 1, 1959.

TREASURER'S REPORT

Financial Statement as of July 7, 1959

Receipts—Academy proper	
Checking account adjusted balance on July 1, 1958 (Security National Bank, Raleigh)	$1691.37
Dues from memberships, regular	1491.04
Dues from patron members	210.00
Dues from sustaining members	625.00
Collegiate Academy gifts	125.00
Collegiate Academy funds deposited	56.84
N. C. Psychological Association, for programs	6.00
Derieux Prize in Collegiate Academy	50.00
Exhibit Fees, Commercial	20.00
AAAS, Research Grant Funds	416.40
National Science Foundation, indirect costs on grant	1301.85
Total Receipts, Academy proper	$5993.50
Receipts—Science Fair Program	
Entry Fees from seven districts	1300.00
Medical Society of N. C.	100.00
Medical Auxiliary, Medical Society of N. C.	25.00
American Chemical Society, N. C. Section	75.00
N. C. Society of Bacteriologists	50.00
Carolina Power and Light Company	175.00
Southern School Supply Company	180.00
J. D. Taylor for Denoyer-Geppert Company	50.00
Carolina Biological Supply Company	50.00
Cardinal Products, Inc.	100.00
N. C. State Fair, Raleigh	492.50
Total Receipts, Science Fair Program	$2597.50
Total receipts for Academy and Science Fair Program	$8591.00

Expenditures—Academy proper	
Derieux Award, Collegiate Academy	$ 50.00
Cancas, Collegiate Journal and other Collegiate Academy expense	161.07
Research Grants made	1521.40
Postage, Stationery, Office supplies	360.09
Expenses for annual meeting	72.85
Printing	143.50
Secretarial aid	100.10
Secretarial commission, 1957–58	135.90
Secretary's travel	39.55
Telephone (long distance calls)	30.31
Expense, representation to AAAS convention, 1958	67.15
Elisha Mitchell Journal	843.00
Academy Conference	20.50
Total Expenditures for Academy proper	$3545.42
Expenditures—Science Fair Program	
Science Fair Manual, 3000 copies	$329.85
Announcement Brochures, 1959 Fairs	180.00
Entrance Fee, National Fair	175.00
To U. N. C., for State Science Fair expense	200.00
Expense for National Fair party, five persons, to Hartford, Connecticut	786.27
Prizes at State Science Fair	220.00
Luncheon and travel for 1960 Fairs planning session	85.78
Travel allowances, N. C. State Fair exhibitors	492.50
Miscellaneous expense	104.17
Total Expenditures for Science Fair Program	$2573.57
Total Expenditures for Academy and Science Fairs	$6118.99
Bank balance as of July 7, 1959	$3779.42
Plus deposits not shown	57.30
	$3836.72
Less outstanding checks	1364.71
	$2472.01
Adjusted bank balance	2472.01
Total to account for	$8591.00
Total Assets	
Checking account, adjusted	$2472.01
Raleigh Savings and Loan Account as of July 7, 1959	360.54
Total Assets	$2832.55

On the 18th of July, 1959, we, the undersigned, examined this account and found it to be correct.

(Signed) W. E. COOPER, *Chairman*
E. F. CANADAY
F. W. LANCASTER

THE POTEAT AWARD

The award for this year was given to the best paper presented before the Geology Section. The award was won by Dr. G. R. MacCarthy of the University of North Carolina for his paper, "The energy released during the formation of joints". A certificate will be presented at the Academy banquet next year, 1960, at the meeting in Greensboro.

REPORT OF COMMITTEE ON RESEARCH GRANTS

The Committee on Research Grants planned its work so that it would be completed just prior to the annual meeting of the Academy.

Last fall, as soon as an estimate could be made of probable available funds, two deadlines for application were set up and advertised. As was expected, the fall date attracted applications primarily from undergraduates who wished to undertake projects during the school year. The spring date, regrettably brought no appeals from undergraduates but did bring a series of good projects from graduate students. Without exception, these latter were faced with field expenses which, apparently, no department could cover.

It was necessary in several instances to reduce the awards somewhat below amounts requested to stay within our limited finances. In general, subsistence and equipment requests were not approved, a policy which we feel we can defend. Transportation was most frequently requested and was generally looked upon favorably within the limits of our finances. Supplies of a special nature were provided in some instances when it was clearly impossible for the department involved to furnish them.

An attempt was made to help every deserving student applicant to get on with his project if it appeared to deserve support. To this extent, we were successful.

That there were no more applications is regrettable. It was especially disappointing not to have more proposals from undergraduates. Considering that almost 100 departments (biology, chemistry, zoology, physics, botany, geology) in every four-year college in the state were notified of the program, the number of applications seems very small.

The total of all awards amounted to $1331.40 and the average was $102.41. Sixteen requests were submitted, one was withdrawn, two were turned down (not because of lack of merit but

because they were contrary to policy), and thirteen awards were made.

ERNEST BALL
M. K. BERKUT
C. E. JENNER
H. J. OOSTING, *Chairman*

REPORT OF THE CONSERVATION-LEGISLATIVE COMMITTEE

The Conservation-Legislative Committee met once during the past year. Further business and communication was carried on by letter and telephone.

The Committee has proposed as its immediate goal the setting aside for preservation of certain areas of natural scientific interest in North Carolina. Natural areas here may be defined as those which represent a unique geological type; those which support a biota representative of pre-Columbian conditions, or nearly so; those which are unique in the state; those supporting rare species; or those supporting species in unusual locations. These natural areas might be set aside by purchase, by deed, by lease, or by long term agreements with the owner. In the Committee meeting and in later conferences with Mr. Tom Morse, Chief of the Division of State Parks of the North Carolina Resources and Development Commission, a list of natural areas suitable for preservation was drawn up. The areas ranged in size from a few acres up to one of several thousand. A priority rating was assigned each area based on the value of the area for scientific study and on the imminence of its destruction. Possible ways and means of accomplishing the preservation of areas was discussed. Various members of the Committee have agreed to investigate certain of the desirable areas and to approach the owners thereof as the first steps in area preservation.

Largely through the efforts of Dr. C. F. Korstian, Dean-Emeritus of the Duke University School of Forestry and Dr. H. L. Blomquist, Professor-Emeritus of the Botany Department of Duke University, the International Paper Company has been induced to sell or deed to some holding agency 7.5 acres of forest in Chatham County near the confluence of the Deep and the Rocky Rivers. This area supports a number of mature and reproducing white pines, *Pinus strobus*. Mr. Paul Harper of the Paper Company surveyed the area and approached the owners of approximately 30 additional adjoining acres of white pine. The 30 acres are owned by Mr. Paul K. Camp and Mr. Harvey L. Paschal. Both gentlemen are in sympathy with the concept of natural areas and with the preservation of the white pine area in particular. They have agreed to lease the land for 50 or 100 years, or they will each deed their holdings to the administering agency with the proviso that the land cannot be sold. Attempts to dispose of the white pine area would cause the land to revert to Mr. Camp and to Mr. Paschal.

A suitable holding and administrative agency is necessary. At a meeting of the Executive Committee of the Academy, April 27, 1959, the Executive Committee decided that the Academy should not be the holding agency, for this would require incorporation of the Academy and the establishment of machinery to accept and administer this area and others which might later be acquired, and would involve the Academy in expenses which it is not now prepared to meet. The Committee suggested that the University of North Carolina might hold and administer the area unchanged for purposes of research on fauna and flora.

The Conservation-Legislative Committee plans to continue its efforts to have set aside natural areas within this state.

WANDA S. HUNTER
MARGARET Y. WALL
ALBERT E. RADFORD
EDWARD M. LOWRY, *Chairman*

REPORT OF THE COLLEGIATE ACADEMY

The Collegiate Academy seems to have reached a plateau as far as membership is concerned. This year we had nine club members, an increase of two, and thirty-three individual members, a decrease of eight, over last year. The increased interest in club memberships reflects, I believe, the lectureship program, which was run by Dr. T. W. Johnson, Jr., and to which many professors at Duke University as well as the three branches of the University of North Carolina contributed. This apparently fills a real need and we hope those involved will be willing to continue.

Since the aim of the organization is to stimulate undergraduate research and to provide opportunity for students to present papers, it was disappointing to have only five papers at the annual meeting, the smallest number since the organization was started. They were:

1. Fossil Deposits of Eastern North America by Harley Hines, Mount Olive Junior College

2. Studies Involving the Thermal Decomposition of Potassium Chlorate by E. P. Mills, Agricultural and Technical College
3. The Effect of X-ray Induced Mutation on the Giant Chromosomes of *Drosophila melanogaster* by Chester C. Haworth, Guilford College
4. The Development of a Streptomycin Resistant and Dependent Strain of *E. coli* by Yancey E. Beamer, Guilford College
5. Morphological Variations in the Periproct of *Arbacia punctulata* (Lamarck) of Beaufort, N. C. by Thomas S. Hopkins, East Carolina College

After the presentation of papers, a business meeting and election of officers was held. Dr. Johnson announced the decision of the judges awarding the Derieux award of $50 for the best paper to Thomas Hopkins, and honorable mention to E. P. Mills and Chester Haworth. He also initiated a new award, a gold key, to be given to the member who had made an outstanding contribution to the success of the Collegiate Academy and presented the first one to the retiring president, Becky Surles.

Before the afternoon meeting the Collegiate Academy members and sponsors met for luncheon and to hear a talk by George A. Herbert, president of the Research Triangle. As in previous years, two issues of Cancas were published, financed by contributions from three chapters of Sigma Xi. In October the meeting of the executive board consisting of the student officers, faculty sponsors, and delegates from ten colleges was held at Meredith College. Plans for the year were discussed.

The officers for next year are: Thomas Hopkins, East Carolina College, president, Chace Collins, Flora Macdonald, vice-president, and Pat Rose, Woman's College, secretary. Faculty sponsors will be John Vernberg, Duke University, lectureship program; Robert Haubrich, East Carolina College, annual meeting; and Charlotte Dawley, Woman's College, treasurer and editor of Cancas. Terry Johnson, Duke University, who has done such a splendid job with both the lectureship program and the annual meeting for the past two years, has resigned, due to other commitments. His enthusiasm and sound judgment will be missed.

Student interest in the Collegiate Academy is increasing, and we hope that faculty members who have students interested in doing original work will take advantage of the opportunities that the Collegiate Academy offers.

CHARLOTTE DAWLEY

NORTH CAROLINA SCIENCE FAIR PROGRAM

This report includes only general statements concerning the operations of the Science Fair Program in North Carolina during the period May 1, 1958, to the present date.

During the year the Institute of Natural Science at the University of North Carolina continued its co-sponsorship of the program. I continued as director. The Institute furnished all secretarial help and handled all mailing in connection with the program. Without the help of the Institute it would have been impossible for me to handle the rapidly expanding correspondence incidental to the operations of the program.

The same general program that has been followed in past years was followed. This included the holding of seven district fairs, the promotion of local fairs, the holding of the state fair, and the sending of two winners to the national fair at Hartford, Connecticut. It should be mentioned at this point that Miss Nancy Green of Enka High School won a fourth place award in the biological division at the 1958 National Fair. The director would at this time like to express to the directors of the district fairs his appreciation for their fine work.

Again there was an increase in local fair participation although it is impossible to obtain figures on local and school fairs. Participation in the district fairs showed a considerable increase although at this time the exact figures have not been obtained from some of the district directors. The State Fair was held at the Planetarium at the University of North Carolina at Chapel Hill. Mr. Jenzano, the Director of the Morehead Planetarium, did a very fine job in handling most of the details of the actual staging of the fair. The Fair Program owes him a debt of gratitude for his fine help. Attendance is estimated to have been between five and six thousand. There was a constant stream of visitors during the entire period of the fair. The time for the state fair was reduced from three to two days. It was felt that this was desirable. It kept the participants and teachers from their school work for a shorter period of time and reduced the housing problem which had become a difficult

one. The exhibits at the state fair were uniformly better than in prior state fairs.

Dr. Yarbrough again did fine work in connection with lining up sponsors and in helping with the finances of the program. The fair program is in good shape financially and a financial statement will be given by Dr. Yarbrough in the November issue of the Journal of the Elisha Mitchell Society.

A final report for the year will be prepared by the director. This will give a list of sponsors, data from the district fairs, and a list of participants and winners in the state fair.

A Science Fair Manual was prepared by the writer, and a sufficient number were printed to last for at least two years. There was a widespread demand for the Manual, and it is felt that it added greatly to the expansion of the program.

H. D. CROCKFORD, *Director*

JUNIOR SCIENCE SYMPOSIUM

The Junior Science Symposium was conducted for the second year by the Office of Ordnance Research and the N. C. Academy of Science. For the first time this year the North Carolina Academy of Science acted as a co-sponsor. Participating in the program were the three major educational institutions of the Research Triangle area: Duke University, North Carolina State College and the University of North Carolina at Chapel Hill. The principal role of the Academy of Science was in helping select the participants. Dr. John Yarbrough served as an active member of the steering committee for the Symposium and handled all details in connection with the selection of the participants. Four hundred and seventy students and teachers from one hundred high schools attended the symposium.

The same program was followed as was used the prior year except for the addition of what was termed a curbstone clinic. Each morning the entire group met at one of the participating schools where three high school students presented papers on a specific scientific project, and a faculty member from the school gave an hour talk on a subject of his choice. The afternoons were given over to research tours on the three campuses. One night was given over to the main speaker, who this year was Dr. Ralph E. Gibson, Director of the Applied Physics Laboratory at the Johns Hopkins University. The curbstone clinic was held the last night in Hill Music Hall at Chapel Hill. This program consisted of several talks on subjects dealing with careers in science. After the formal talks a series of questions from the floor were answered by the members of the panel. This program was one of the high lights of the symposium.

J. A. YARBROUGH

REPORT OF FIFTH ANNUAL NORTH CAROLINA SCIENCE TALENT SEARCH

Five North Carolina entries were awarded Honorable Mention in the National Westinghouse Science Talent Search and were declared winners in the North Carolina Science Talent Search. Twelve others were awarded Honorable Mention. The selection committee consisted of Arthur Kelman, B. B. Brandt, Henry Shannon and Herbert Speece.

Nine entrants were invited to present papers at the Junior Science Symposium and three more have been invited to present papers at the Academy meeting. These were selected on the basis of over-all score and/or an outstanding project or research.

By official tabulation[1] Science Clubs of America states that 93 schools requested 698 examinations and entry blanks but only 60 completed entries were returned, as compared with 650 requested and 82 completed last year.

Congratulatory letters were sent to all winners and honorable mentions. A letter of commendation has been sent to the teachers who filled out and signed the entries and the principal of their schools. It is planned to send letters to all those teachers who requested examinations but who had no students completing an entry, commending them for their interest and suggesting they begin now to plan entries for next year.

HERBERT E. SPEECE, *Director*

REPORT OF THE ACADEMY CONFERENCE

The 1958 meeting of the Academy Conference was held during the AAAS meetings in Washington, D. C., on December 28, 1958. John A. Yarbrough and T. W. Johnson, Jr. represented the N. C. Academy of Science. Dr. Yarbrough presided over the 1958 session as Academy Conference President. The morning session was taken up in routine business and reports from

[1] By actual count from lists furnished by Science Clubs there were 868 examinations requested by 117 schools in North Carolina.

standing committees. The AAAS reported that for 1958 research allotments to each participating Academy would be on the basis of $1 per member who is also a member of AAAS. This represents a doubling of this allotment. Prominent in the morning discussion were remarks from Dr. W. J. Peterson of the National Science Foundation on how Academies could submit proposals for science education projects to NSF. More than thirty Academies have now taken advantage of this opportunity. Representatives from various Academies reported as usual on outstanding activities of their respective Academies during the year 1958. New officers elected were: Dr. John G. Arnold, Jr. of the New Orleans Academy as President-elect for 1959; Dr. E. Ruffin Jones of Florida Academy as Secretary-Treasurer. Dr. A. M. Winchester of Florida will serve during 1959 as President of the Conference.

The afternoon session consisted of two concurrent programs, the first on problems of the Senior Academies and the second on problems of Junior Academies. Dr. Winchester was the leader of the first and Miss Elnore Stoldt of Illinois was chairman of the latter. The usual evening banquet session was presided over by Mrs. Thelma G. Heatwole, retiring President. President John A. Yarbrough presented his address on the topic "A new day for science?"

Report on Council Meetings of the AAAS

Two meetings of the Council of the AAAS were held in Washington on December 27 and December 30, 1958. It was reported that three officials had been previously elected by mail ballots of the Council members. They are Chauncey D. Leake for President-elect of the AAAS and Bentley Glass and Margaret Meade on four-year terms on the Board of Directors. The Council voted to continue and strengthen the work of the Committee on the Social Aspects of Science. An important new step taken by the Council was the appointment of a special committee to study ways in which the Council itself can function more effectively both during the annual convention as well as during the interim between conventions. The Council is a large body consisting of more than 400 members but it wishes to contribute more than it has in the past in the operation of the business of the AAAS. The Council voted to commend Federal Aid to Education as shown in the National Defense Education Act of 1958 and to express its hope that this might continue. Hope was also voiced that the Geneva Conference on control of nuclear weapons tests will eventually bring successful results. John A. Yarbrough represented the Academy at both Council meetings.

Report of the Resolutions Committee

1. A resolution concerning teacher load in the colleges and universities: In determining the teaching load for laboratory work wherein the teacher is in direct charge and is constantly and solely occupied in laboratory instruction, it is recommended that each contact hour be considered the equivalent of an hour of lecture or recitation. It is further recommended that the sixteen hour maximum load mentioned in Standard Five of the Southern Association of Colleges and Secondary Schools be construed to mean sixteen contact hours whether in lecture, recitation or laboratory when the instructor is actually present in the laboratory.

It is further recommended that copies of this resolution be forwarded to Dr. Thelma Howell, Wesleyan College, Macon, Georgia, and to appropriate officials of the Southern Association of Colleges and Secondary Schools.

2. Be it resolved that the North Carolina Academy of Science express its appreciation to President A. R. Keppel, to the faculty and to the students of Catawba College for their cordial hospitality at this the 56th annual meeting of the Academy. To the chairman and members of the local committee on arrangements and to the numerous persons who have cooperated with them we are grateful for the foresight and diligent efforts which have made this meeting proceed smoothly and comfortably in most pleasant surroundings. Be it further resolved that a copy of this resolution be incorporated into the minutes of the Academy for 1959 and that copies be sent to President Keppel and to the chairman of the Committee on Arrangements at Catawba College.

W. O. Puckett

The above resolutions were unanimously adopted by the Academy.

Memorials were read to the following members: Frank Kenneth Cameron and John Simcox Holmes. The memorials were approved by a rising vote of the members.

Frank Kenneth Cameron

Dr. Frank K. Cameron was born in Baltimore, Maryland, on February 2, 1869. He entered the

Johns Hopkins University in 1887, received the A.B. degree in 1891, and the Ph.D. degree in 1894.

After serving on the faculty at Cornell University and Catholic University he became associated with the United States Department of Agriculture in 1898. This association continued for 17 years, and the work done during this period established the direction and the area which were to receive his greatest interest and direct the major portion of his future investigations. From 1915 to 1926 he served as a consulting chemical engineer, mainly in connection with mining operations in the western United States. In 1926 he joined the faculty of the University of North Carolina, where he remained until his retirement in 1946. Following his retirement, he resided in Chapel Hill until his death on August 18, 1958.

Dr. Cameron was a member of the American Chemical Society, the Chemical Society (London, England), the Elisha Mitchell Scientific Society, the Society of Electrochemistry, the American Institute of Chemical Engineers, and the Society of the Sigma Xi. He was a fellow of the Association for the Advancement of Science. He was a member of the North Carolina Academy of Science from 1926 until his death.

Dr. Cameron's research interests were many and varied; the problems of agriculture, development of natural resources, soil chemistry, physical chemistry—especially colloid chemistry and the study of heterogeneous equilibria—mining and metallurgy, textile chemistry, and the chemurgy of the whole cotton plant. In recognition of his many contributions to pure and applied chemistry, he was awarded the Herty Medal in 1939.

Although Dr. Cameron was absorbed in his interest in chemistry, he was an exceedingly warmhearted, friendly, and congenial man. He possessed a keen sense of humor and was held in high esteem by his associates, both faculty and students alike. He was among the early leaders in the Catholic community at Chapel Hill and was one of those influential in the planning and erection of the Chapel of St. Thomas More at that location.

H. D. CROCKFORD

JOHN SIMCOX HOLMES

By the death of J. S. Holmes on May 2, 1958, the nation and North Carolina lost a pioneer in modern forestry. For thirty-seven years he served his adopted State with quiet diligence and fortitude. His coworkers were inspired by his modesty and humility whether he had achieved success or temporary setbacks. He was a man of deep religious nature which was manifested in his Church and in his love for mankind and all other living animals and plants of the outdoors. He was a keen observer and his fellow workers treasured the opportunity of being in the outdoors with him. Even in later years he maintained a physical condition that was envied by younger men.

Mr. Holmes was born in Coburg, Ontario, Canada, where his parents were English settlers a year before. In his third year his parents returned to England and he received his primary education in England and North Wales. At the age of thirteen he again came west with his family who settled on the French Broad River in western North Carolina. Farming was the means of livelihood. John's formal preparatory school work was one year in Wales and four months in Asheville. Nevertheless he entered the University of North Carolina in August, 1886, and took the two-year course for "A Certificate in Agriculture". The farming by the French Broad River, with destructive flooding, was not a success.

At the age of 34, and seeking a livelihood elsewhere, Mr. Holmes secured a student assistant appointment with the U. S. Bureau of Forestry in 1902. His first work was in Texas on a valuation survey and counting the age of longleaf pine stumps.

Following a year of this experience he entered Yale University Forest School and finished the work for the M.F. Degree in 1905. This degree was withheld for ten years because he had no previous college degree. The next four years were spent with the U. S. Bureau of Forestry in the Southwest. By June, 1909, W. W. Ashe was leaving the State Forestry work at Chapel Hill, and "Jack" Holmes was invited to become his successor. The work that year led to the publication of Bulletin 23, "Forest Conditions of Western North Carolina."

With this beginning Mr. Holmes served as State Forester for thirty-seven years. Much of his effort was in a see-saw battle to establish forests as a valuable resource and to secure the legislation and public education necessary to insure the wise use of this resource. When he retired to an emeritus position in 1945 there were tangible and growing results of his diligent efforts. Millions of North Carolina acres are under fire

control, and forestry products are high among our resources. A dozen State Parks and three forest tree nurseries attest to the foresight of "Jack" Holmes.

Colleagues of Mr. Holmes recognized him and he was a Fellow of the Society of American Foresters, President of the Association of State Foresters and President of the Southern Forestry Congress. He was a versatile writer and produced many scholarly publications as well as scores of educational articles.

A creed stated by the deceased was: "I have found that happiness depends not on 'the abundance of things that a man hath'. In public work I have found that it is the best plan to accept responsibility, never pass the buck, and let the other fellow have the credit. In private life, select the right wife and friends and you will be happy".

JOHN SIMCOX HOLMES

HARRY T. DAVIS

REPORT OF THE NOMINATING COMMITTEE

The committee, consisting of E. Willard Berry, W. O. Puckett and H. R. Totten submitted the following nominations:

President: John F. Dashiell, Wake Forest College

Vice-President: Wanda S. Hunter, Duke University

Member of Executive Committee: Jesse P. Tyndall, Atlantic Christian College

Member of Research Grants Committee: John M. Parker, III, N. C. State College

The above nominees were unanimously elected by the Academy. This concluded the business and the meeting was adjourned.

More than 125 members of the Academy, guests, and friends enjoyed the Annual Dinner of the Academy in the dining hall of Catawba College at 7:00 p.m. Vice-president L. A. Whitford presided at the dinner and the brief session which followed. The 1958 Poteat Award, presented annually by Phipps & Bird, Inc. of Richmond, Virginia, was formally presented to E. C. Maine for his paper in 1958 in the Botany Section on "Possible mechanisms of resistance in solanaceous plants to *Pseudomonas solanacearum*." For the third year the John Bewley Derieux Memorial Award was given to a paper chosen as the best presented in the Collegiate Academy session. The presentation for 1959 was made to Thomas S. Hopkins of East Carolina College for his paper "Morphological variations in the periproct of *Arbacia punctulata* (Lamarck) of Beaufort, N. C." Professor R. M. Helms, acting for the Physics Section of the Academy, presented four persons to receive awards as outstanding teachers of high school physics in North Carolina high schools. These were: Miss Estell McClees, Grainger High School, Kinston; James N. Smith, Jr., New Bern High School; John N. Scott, East Mecklenburg High School, Charlotte; and Robert B. Westbrook, Statesville Senior High School. Each of these four teachers will receive honorary membership in the North Carolina Academy for one year. Unfortunately only one of the four could be present at the banquet but each had previously been notified of his selection and arrangements had been made for suitable ceremonies at the home high school of each person.

Following the banquet, the members and friends adjourned to the Main Auditorium of the Administration Building where President MacCarthy presented a Presidential Address on "What, when and why of earthquakes". Vice-president Whitford presided and presented Dr. MacCarthy to the audience. Following the

lecture all members and guests were entertained at a very pleasant social hour through the courtesy of Catawba College.

The following officers were elected for the respective sections:

Biochemistry and Physiology: Chairman, John H. Ferguson; Secretary, Dr. Maude F. Williams

Botany: Chairman, Victor M. Cutter, Jr.; Secretary, H. B. Howe

Geology: Chairman, James F. Conley; Secretary, S. Duncan Heron, Jr.

Mathematics: C. G. Mumford; Secretary, L. L. Garner

Physics: Chairman, R. M. Helms; Secretary, Thomas F. Turner

Zoology: Chairman, C. G. Bookhout; Secretary, C. M. Allen

The following constitute the personnel of the standing committees:

Executive: John F. Dashiell, Wanda S. Hunter, John A. Yarbrough, Ben H. Hill, Paul J. Kramer, and Jesse P. Tyndall

Research Grants: Ernest Ball, C. E. Jenner, M. K. Berkut, and John M. Parker, III

At this writing Dr. Dashiell, President of the Academy has appointed the Auditing Committee consisting of W. E. Cooper, E. F. Canaday and F. W. Lancaster. Other committees will be appointed in the early fall of 1959 for the school year of 1959–60.

The following papers were presented during the meetings at Catawba College. Those marked with an x are abstracted in these proceedings:

GENERAL SESSION

Some medical problems in manned space flight. Dr. Edwin P. Hiatt, Chief, Biophysics Branch, Aero Medical Laboratory, Wright-Patterson Air Force Base, Ohio

COLLEGIATE ACADEMY

Fossil deposits of eastern North America. Harley Hines, Mount Olive Junior College

Studies involving the thermal decomposition of potassium chlorate. E. P. Mills, Agricultural and Technical College

Paramagnetic resonance. Marvin Swartz, Wake Forest College

The effect of x-ray induced mutation on the giant chromosomes of Drosophila melanogaster. Chester C. Haworth, Guilford College

The development of a streptomycin resistant and dependent strain of E. coli. Yancey E. Beamer, Guilford College

Morphological variations in the periproct of Arbacia punctulata *(Lamarck) of Beaufort, N. C.* Thomas S. Hopkins, East Carolina College

BIOCHEMISTRY AND PHYSIOLOGY SECTION

x *Processes related to Hageman factor activation.* Charles L. Johnston, Jr. and John H. Ferguson, U.N.C.

x *A rapid complexometric titration of serum calcium.* H. C. McAllister, Jr., and C. L. Yarbro, U.N.C.

x *The comparison of aerobic and anaerobic glycolysis in the liver tissue of developing chick.* Yoon T. Kim and D. G. Humm, U.N.C.

x *Serotonin response as an index to radiation recovery in irradiated mice.* Gladys W. Royal and George C. Royal, A. and T. College of N. C.

BOTANY SECTION

x *The sexual stage of* Chytriomyces. William J. Koch, U.N.C.

x *Cylindrocladium root rot of yellow poplar.* A. Kelman, G. V. Gooding, and G. K. Slocum, N. C. State

Infection of barnacle ova by a Lagenidium. T. W. Johnson, Jr., Duke University

Some unusual fresh-water diatoms in North Carolina. L. A. Whitford, N. C. State

x *Yellow poplar dieback.* Robert W. Powell, Jr., Duke University

x *Studies on the translocation of minerals and carbon compounds between corn and* Striga asiatica. W. E. Rogers and R. R. Nelson, N. C. State

x *Notes on the structure and origin of the ovule in the genus* Ilex *L.* J. M. Herr, Jr., Pfeiffer College

A time-lapse study of the growth of the shoot apex of Lupinus albus. Ernest Ball, N. C. State.

An interesting plant community in the Piedmont of South Carolina. Albert E. Radford, U.N.C.

A floristic study of a sandhills area on the North Carolina coastal plain. C. John Burk, U.N.C.

x *Better trained science teachers for our high schools—a desperate need.* Elton C. Cocke. Wake Forest College

GEOLOGY SECTION

x *A banded agate locality in the North Carolina Triassic.* James F. Conley, N. C. Depart-

ment of Conservation and Development, Division of Mineral Resources

x *Granite in eastern Wake County, N. C.* Ernest M. Dumas, N. C. State.

x *Alumina content of some residual soils in the Piedmont of South Carolina.* Richard J, Councill and Clement M. Llewellyn, Jr. Atlantic Coast Line Railroad Company

x *Origin of ground water in the Triassic basin of North Carolina and Virginia.* Harry LeGrand, Consulting Geologist

x *Some Cretaceous Foraminifera and Ostracoda from Kinston, N. C.* Marcus B. Morehead, U.N.C.

Tectonic process as exemplified by the North Carolina-South Carolina salient of the Appalachian orogenic belt. James W. Clark, Duke University

The energy released during formation of joints. G. R. MacCarthy, U.N.C.

The development and use of geology in the North Carolina Highway Commission. A. C. Dodson, State Highway Commission

x *Ecologic and faunal analyses of some fossil assemblages of the Cretaceous Black Creek and Peedee Formations.* C. Everett Brett. U.N.C.

x *Dunite bodies at Holcombe Branch and Democrat in Madison and Buncombe Counties,* N. C. James A. Ray, N. C. State College

x *The sediments of the Beaufort Inlet area, N. C,* R. Wesley Batten and Roy L. Ingram. U.N.C.

MATHEMATICS SECTION

Parallel spirals. T. F. Hickerson, U.N.C.

On a paper of L. Guttman, A. C. Mewborn, U.N.C.

x *Hausdorff transforms of bounded sequences.* J. H. Wells, U.N.C.

x *A canonical form for certain osculants.* J. W. Lasley, Jr., U.N.C.

x *A matric representation of complex numbers.* H. M. Nahikian. N. C. State

x *An angle relation associated with parabolas.* L. L. Garner, U.N.C.

PHYSICS SECTION

x *Effects of heat treatment and mechanical deformation on dark conductivity of AgCl crystals.* Howard P. Layer, U.N.C.

Lattice dynamics of body centered cubic crystals. D. N. Singh, U.N.C.

Increasing the effectiveness of demonstrations and laboratory in Introductory Physics, Joseph Ward Straley, U.N.C.

Diffusion of radio-tracers in crystals of gold-silver alloys. Ralph Bass, U.N.C. (introduced by L. Slifkin)

x *General nuclear reactor control rod theory.* Raymond L. Murray, N. C. State.

x *Calculation of effects of burnup on reactor properties.* Syed Ahmed Hasnain, N. C. State.

Non-uniform nuclear reactors. David A. McCutchan, N. C. State.

x *Transient heat transfer in reactor coolant channels with thin fuel elements.* E. L. Arnold and W. O. Doggett, N. C. State.

x *A time correlation technique for investigating three successive nuclear transformations.* W. O. Doggett and T. E. Todd, N. C. State

Nuclear propulsion for space travel. Dr. A. C. Menius, N. C. State. (invited paper)

ZOOLOGY SECTION

Carrot rust fly damage to different host plants. H. E. Scott, N. C. State

Resistance of vegetable varieties to insects. Charles H. Brett, N. C. State

x *The genitalia of Elateridae (click beetles).* Walter M. Kulash, N. C. State

x *Hieroglyph, or gravity aligned steering substance in bee dance?* A. L. Pickens, Alderson-Broaddus College

x *Observations on the spawning and early development of the common scallop,* Pecten irradians. L. M. Outten, Mars Hill College

x *Differentiation and proliferation in the development of amphibian adrenals.* Laura G. Anderton, W. C., U.N.C.

x *The effects of age on hyperplasia in the remaining rat kidney following unilateral nephrectomy.* Charles E. McCreight, Bowman Gray School of Medicine

x *Effects of temperature and salinity on the development of* Axiothella mucosa (*Andrews*). Murad-Baba Murad Mohammad, Duke University

x *The use of radioactive gold (Au) to trace movements and field activity in Eastern harvest mice,* Reithrodontomys humulis humulis (*And. and Bach*). Stephen V. Kaye, N. C. State

HIGH SCHOOL TEACHING SECTION

Effect of temperature and normality changes on electrolysis of certain chloride salts. Andrew

D. Rogers, Needham B. Broughton High School, Raleigh

Utilizing solar power. Frederick C. Wedler, Greensboro Senior High School, Greensboro

Experiments in entomology. Betty Lou Wallace, Mountain View High School, Thurmond

Science Fairs sponsored by the Academy of Science. H. D. Crockford, U.N.C.

The Westinghouse Talent Search and the Junior Science Symposium. Herbert E. Speece, N. C. State

Science clubs at Daniels Junior High School. Eugene Upchurch, Daniels Junior High School, Raleigh

A high school teacher looks at physics. Clarence Shipton, Curry High School, Greensboro

x *Expanding student horizons through science roundtables.* James W. Manley, Director, Charlotte Nature Museum, Charlotte

Processes related to Hageman factor activation. CHARLES L. JOHNSTON, JR. AND JOHN H. FERGUSON. "Clot-promoting activity" is developed in plasma that has been exposed to surface contact. Evidence was presented at the 1958 Academy meeting which showed the primary role of Hageman factor in this process. These findings will be reviewed briefly, and new studies presented to show the relationship of inhibitory phenomena to Hageman factor activation.

Plasmas were activated by crushed glass and the partial thromboplastin time test used for assay purposes. Once initial plasma contact had been established, no essential difference was noted in results of tests performed in glass or silicone.

Greater clot-promoting effect develops in normal and Hageman deficient plasmas exposed to contact separately, then mixed, and tested, than when they are mixed, contacted together, and then tested. This confirms the finding of others that Hageman deficient plasma contains an inhibitor which tends to oppose the activation of Hageman factor. In addition, it is shown that inhibition of normal plasma does not result until the concentration of Hageman deficient plasma is present in excess relative to normal plasma. Thus, the inhibitory effect is weak. However, it may be potentiated by incubation.

Normal plasma exposed to contact, then removed from further contact, gradually loses its clot-promoting effect. This has been called "decay". New data suggest that this phenomenon is a progressive inactivation of the Hageman factor itself and not due to depletion of any other clotting factors.

On the basis of the results, it is possible to postulate that two inactivation mechanisms are related to the Hageman factor. One tends to oppose Hageman factor activation and is removed by contact. The other, apparently not affected by contact, seems progressively to inactivate the "active" Hageman factor produced.

At the present time, the relationship of these surface reactions to the clotting mechanism is obscure. The demonstration that such are related to *in vivo* fluidity or clotting of blood must await future investigations.

A rapid complexometric titration of serum calcium. H. D. MCALLISTER, JR. AND C. L. YARBRO.[2] The procedure of Yarbro and Golby (Anal. Chem. *30*, 504, 1958) for the determination of urinary calcium has been adapted for the determination of serum calcium on 0.2 to 2.0 ml. aliquots of serum. The method consists of addition of cyanide to complex ferrous iron, sodium hydroxide to adjust pH to the range of 12.0 to 12.5, followed by EGTA [ethylene glycol bis-(B-aminoethyl ether)N,N′tetraacetic acid] in excess. The excess EGTA is back-titrated with standard calcium by means of a microburet using Calcon [1-(2-hydroxy-1-naphthylazo)-2 naphthol sulfonic acid] as an indicator. No interference was noted from ferrous iron, magnesium, citrate or phosphate. Recoveries of calcium added to serum samples ranged from 97 to 103 per cent. Results obtained with this procedure compared favorably with those obtained from standard clinical procedures where calcium is isolated as the oxalate. Replicability among identical aliquots was on the order of 2 to 4 per cent.

The comparison of aerobic and anaerobic glycolysis in liver tissue of developing chick. YOON T. KIM AND D. G. HUMM. The aerobic and anaerobic glycolysis were compared in the liver tissue homogenate of chick embryo from 6th to 20th day of incubation during development, young chick from the time of hatching to 30 days, and one year old adult chick, employing

[2] Supported in part by a research grant (PHS A-248) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.

the procedure devised by LePage et al. (1949, 1953).

From the results in this present work, it has been demonstrated that glycogen could be phosphorylated during glycolysis by liver tissue of chick embryo.

In the comparison of aerobic and anaerobic glycolysis of liver tissue, the rate of anaerobic glycolysis was higher than that of aerobic glycolysis. The rate of anaerobic and aerobic glycolysis and the ratio between anaerobic and aerobic glycolysis were higher in liver of embryo and young chick than those in adult chick. And in measurement of aerobic glycolysis, the possibility of Pasteur effect has been observed in both embryonic and adult liver tissue in the presence of oxygen.

The rate of glycolysis was increased from 6th day of incubation, reaching its maximum at around 12th day, after which it was decreased. In the young chick, the rate of glycolysis was increased until about 2 weeks after hatching and then decreased again gradually until the young chick was one month old. Under the connection of the experiment of glycolysis on 12 month old adult chick with the previous experiment on 1 month old chick, it would be assumed that there is a general tendency of decrease in its rate.

From these results, the possible assumption on two maximal peaks of glycolytic rate was made that they are associated with the increment of functional activities of liver tissue of embryo and young chick at those periods.

Serotonin response as an index to radiation recovery in irradiated mice. GLADYS W. ROYAL AND GEORGE C. ROYAL. The increased infiltration of organic dyes following a subcutaneous injection of serotonin can be quantitated in ears of mice. BDF_1 females show increased sensitivity to serotonin injections after total-body exposure to 900 r of X-irradiation and a protective dose of heterologous (rat) bone marrow. Viscosity studies with total-body mouse homogenates show that such animals also have increased fluidity in their composite tissues. The serotonin response per unit body weight, when correlated with mortality rate provides a characteristic value for animals that survive the treatment 30 days or more. Application of this relationship allows the prediction of survivals in a sampling of test animals. Such an index offers increased efficiency in experimental design necessary to explain erratic success with rat bone marrow treatments in irradiated mice. Since the method requires neither expensive equipment nor animal sacrifice; is sensitive, specific, and reproducible; its utilization is considered practical.

The sexual stage of Chytriomyces. WILLIAM J. KOCH.[3] *Chytriomyces* was described by J. S. Karling in 1945 to accommodate two chitinophilic species, *C. aureus* and *C. hyalinus*. In one of my collections at Highlands last summer, the resting spores of what is presumed to be *C. hyalinus* were observed. On chitin they are extramatrical and they appear to be asexual and formed in the same way as zoosporangia; however, observations of agar cultures reveal that the resting spore thalli form sexually at the tips of rhizoids from two contributing thalli. In the type species and in all but one of the subsequently described species, resting spores have been reported. In each, resting spore formation has been assumed to be asexual and with early stages like those of zoosporangia, but in no species have these features been shown convincingly. It may be that the sexual or apomictic formation of resting spore thalli at the tips of rhizoids is a definitive feature of the genus.

Cylindrocladium root rot of yellow poplar (Liriodendron tulipifera *L.*) ARTHUR KELMAN, G. V. GOODING, JR. AND G. K. SLOCUM. Cultures of *Cylindrocladium scoparium* Morgan were obtained consistently in isolations from diseased roots of yellow-poplar seedlings grown in a forest tree nursery in North Carolina. Greenhouse inoculations were made using isolates of *C. scoparium* from yellow-poplar as well as isolates obtained from eastern white pine in North Carolina and Delaware, and from azalea in Alabama. Rapid death of 8-week-old yellow-poplar seedlings followed root inoculations. Girdling lesions developed at the base of the stem of inoculated 2-year-old seedlings followed by gradual wilting and death over a period of 6 to 8 weeks. Isolates of *C. scoparium* from yellow-poplar, white pine and azalea were also pathogenic to eastern white pine and Fraser fir in greenhouse inoculations. The latter species had not previously been reported as a host for this pathogen. In field studies roots of one-year-old yellow-poplar seedlings from 3 forest tree nurs-

[3] A National Science Foundation Grant-in-aid administered by the Highlands Biological Station is gratefully acknowledged.

eries in North Carolina were dipped in mycelial suspensions of *C. scoparium* prior to planting in replicated blocks. After one year only 43% of the inoculated seedlings had survived compared to 84% of the non-inoculated seedlings. All inoculated living plants that were sampled showed evidence of root infection. Yellow-poplar seedlings with root lesions obtained from a nursery in which *C. scoparium* had caused heavy damage on eastern white pine were also planted; after one year only 51% of the naturally infected seedlings were alive. Re-isolations from roots of inoculated and naturally-infected yellow-poplar seedlings yielded typical cultures of the pathogen.

It is suggested that the failure of plantings of yellow-poplar which have been common in North Carolina may be attributed in part to use of seedlings with root infections of *C. scoparium*.

Yellow-poplar dieback. ROBERT W. POWELL, JR. Yellow-poplar dieback is an apparently enphytotic disease of *Liriodendron tulipifera* L. that has been reported from Mississippi, Louisiana, Alabama, Tennessee, and North Carolina. The dieback is prevalent on saplings and young trees and is expressed symptomatically as dying back of branches, formation of branch and trunk cankers, chlorotic atrophism of leaves, sparse crown, and often epicormic branching. Isolations from infected trees in the Duke University Forest have yielded a *Dothiorella* sp. that will produce symptoms when inoculated on healthy yellow-poplar seedlings. It is believed that this *Dothiorella* sp. is the imperfect stage of *Botryosphaeria ribis*, a known plant pathogen on a wide variety of hosts.

Studies on the translocation of minerals and carbon compounds between corn and Striga asiatica. W. E. ROGERS AND R. R. NELSON. Histological studies of the host-parasite relationships of corn and the phanerogamic parasite, *Striga asiatica*, were designed to determine the cellular pathway of translocated compounds. With the use of the rotary microtome, the freezing microtome, macerations with H_2O_2 and autoradiograms, it was found that the phloem is well developed in the stems of witchweed plants, but efforts to identify phloem tissue in the haustoria were inconclusive. Xylem tissue appears to be the important means of connection between host and parasite.

Investigations with radioactive phosphorus and sulphur demonstrated that these minerals were translocated to the parasite when fed either to the roots or leaves of corn. Translocation occurred rapidly and only in the direction of host to parasite. There appeared to be a greater accumulation of translocated materials in the leaves rather than the stems or fruits of the parasite. Studies with radioactive glucose, introduced into cut tips of corn leaves, indicated a greater accumulation of C^{14} in the parasite than in host roots. Similar results were obtained with $C^{14}O_2$. Supplementary studies with C^{14} indicated that the chlorophyll present in the parasite is functional.

Notes on the structure and origin of the ovule in the genus Ilex *L.* J. M. HERR, JR. Ontogeny of the ovule has been studied in six species from the principal subgenera of the genus *Ilex* L. Continuous features of structure are axile placentation, anatropous form, apical lignification of the single integument, characteristic cell differentiation in the epidermal and tapetal layers, and the presence of a protuberance on the inner surface of the funiculus. The funicular protuberance, regarded by Loesner (1942) as a reduced homologue of the Cassinoidean (Celastraceae) aril, occasionally in *I. crenata* Thunbg. and *I. vomitoria* Ait. develops into a second ovule. This event is regarded as evidence that the *Ilex* ovule is a secondary structure formed by the coalescence of two primary ovules. The primary ovule of Angiosperms is defined, and on the basis of evidence from the literature its origin through the coalescence of two or more megasporangia is proposed.

Better trained science teachers for our high schools—A desperate need. ELTON C. COCKE. A study of the records of the high school science and mathematics teachers who made application to attend the National Science Foundation Summer Institute at Wake Forest College showed that for the most part they are poorly trained in their subject. Less than twenty percent of the applicants majored in the subject which they are teaching. It appears that the best trained college graduates who majored in the sciences or mathematics accept positions in other fields where the financial remuneration is more attractive. This has forced the public school administrators to accept teachers to teach the sciences and mathematics who are poorly trained. To say the least, this is regrettable.

A banded agate locality in the North Carolina Triassic. JAMES F. CONLEY. A banded agate and chalcedony locality in Triassic rocks was recently brought to the writer's attention by Mr. J. Alton Price, Durham, North Carolina. Chalcedony and agate occur in the fields of the Whitfield brothers' dairy farm, north of Creedmoor, Granville County. A thin chalcedony bed is found in place to the east, in an exposure on Highway U. S. 15.

Specimens of chalcedony and agate from the farm are tabular masses and many are hollow inside. Others are lined with quartz crystals pointing toward the center, indicating growth in this direction. To account for this phenomenon, deposition had to occur in open cavities. Rhombic impressions on the exterior of the specimens indicate silica replaced or dissolved out a rhombic mineral, probably calcite.

The chalcedony exposed on Highway U. S. 15 is part of a stratigraphic sequence consisting of arkose overlain by a thin clay bed. The chalcedony lies on top of the clay and in turn is overlain by coarse sandstone. This material does not contain hollow centers or rhombic impressions on its outer surfaces.

It is postulated that groundwater charged with colloidal silica percolated downward filling vugs, once occupied by calcite nodules, with chalcedony and agate. The chalcedony found on the road cut had a slightly different origin. The downward percolating groundwater, impeded in its forward progress by the underlying clay bed, deposited silica in the form of chalcedony on top of this clay bed.

Granite in eastern Wake County, North Carolina. ERNEST M. DUMAS. A portion of a large granitic pluton located in the eastern Piedmont area of North Carolina is being investigated with the intention of describing the character of the granite and, if possible, arriving at some conclusion as to the mode of origin. Within the two mile wide traverse being studied, the vague and ill-defined relationships at the border contact areas, the association with regionally metamorphosed gneisses and schists, and the frequent occurrence of well-foliated inclusions within the more massive granite tend to favor a metamorphic rather than an igneous origin for the granite. From a thin-section study of the granitic material, it appears that the term quartz monzonite is more applicable to this particular body than the term granite. Lack of zoning in plagioclase grains and the rather uniform composition of the plagioclase, $Ab_{78}An_{22}$, seem to be in agreement with a metamorphic origin for the area studied. Saprolite panning and heavy mineral separation are being done with the primary purpose of ascertaining the distribution of the mineral monazite, which has been reported from this area. High level sediments, which appear to cap most of the topographically high areas, are being mapped.

Alumina content of some residual soils in the Piedmont of South Carolina. RICHARD J. COUNCILL AND CLEMENT M. LLEWELLYN, JR. In cooperation with a major aluminum producer, samples of residuum were collected by Industrial Geologists of the Atlantic Coast Line Railroad Company in selected localities in the Piedmont of South Carolina between Columbia and Greenville-Spartanburg and Calhoun Falls and Anderson for analyses to determine free alumina content. A total of 99 samples obtained from residuum developed on various types of mica schists, rocks of the Volcanic-slate series, granite, granite with pegmatite, sillimanite schist and hornblendite contained free alumina in amounts ranging from 2 to 16 per cent and iron oxides in amounts ranging from 2 to 11 percent. Total alumina ranges from 8 to 26 percent. Free alumina is in the form of the mineral gibbsite ($Al_2O_3 \cdot 3H_2O$).

Based on the information available from the field and laboratory work, residuum from sillimanite schist and hornblendite, respectively, contain on the average the greatest amount of free alumina and in general the smallest amount of total alumina. Because hornblendite is more abundant than sillimanite schist in the climatologically favorable Spartanburg County area, considerable exploratory work has been conducted by several aluminum producers in parts of the county underlain predominantly by hornblendic rocks, but no commercial or marginal deposits of gibbsite-bearing residuum have been located. However, additional work is planned by the authors as a matter of personal interest.

Origin of ground water in the triassic basins of North Carolina and Virginia. HARRY E. LEGRAND. The present ground-water circulatory system in the Triassic basins of North Carolina and Virginia represents a zone composed of loose, weathered material and interconnecting openings along bedding planes and joints. This zone in most cases does not extend more than 100 feet below the ground. In spite of shallow occurrence

in non-marine shales and sandstones, much of the water is mineralized.

The geologic and hydrologic history of the rocks has a bearing on the present character of the water. The original water carrying the sediments into the basins contained very little dissolved mineral matter. Much of the water was buried with the sediments and a high percent was lost by evaporation, but the buried ground water had extremely poor discharge facilities. The tendency toward concentration of soluble matter continued with diagenesis. Compaction of the thick sedimentary series resulted in reduction in pore space and an upward escape of ground water. The dissolved mineral matter, chiefly salts of sodium, calcium, and magnesium, were ultimately precipitated as cementing material and as fillings along some bedding planes, faults, and joint systems. These salts that occur in the present ground-water circulatory system became redissolved and represent the mineralized water typical of Triassic rocks of the two States.

Very little dissolved material has ever left the basins and much may have been precipitated in concentrated amounts by upward movement of ground water during diagenesis. The chance for economic accumulations of uranium and lesser known or exotic elements appears good enough to merit intensive investigation.

Some Cretaceous Foraminifera and Ostracoda from Kinston, North Carolina. MARCUS B. MOREHEAD. A Cretaceous (Peedee) locality, located .5 miles West of Kinston, North Carolina, on the North bank of the Neuse River was investigated for its foraminiferal and ostracod content.

The following species of Foraminifera and Ostracods were identified and are listed in their order of abundance: *Foraminifera. Robulus pseudosecans* Cushman; *Robulus spisso-costatus* Cushman; *Guttulina adhaerens* (Olsewski); *Bulliminella carseyae* Plummer; *Anomalina ammonoides* (Reuss); *Anomalina nelsoni* Berry; *Globigerina cretacea* d'Orbigny; *Globigerina* (*Globigerinella*) *planispira* Tappan; *Planulina taylorensis* (Carsey); *Nodosaria affinis* Reuss; *Nodosaria obscura* Reuss; *Dentalina* (*Nodosaria*) *gracilis* (d'Orbigny); *Robulus aldrichi* Sandrige; *Buliminella vitrea* Cushman & Parker; *Bulimina prolixa* Cushman & Parker; *Globotruncana fornicata* Plummer; *Planulina texana* Cushman; *Gümbelina reussi* Cushman; *Gaudryina rudita* Sandrige; *Dorthia conulus* (Reuss); *Pyrulina cylindroides* Cushman & Ozawa; *Clavulinoides asper* (Cushman) *Raphanulina* (*Globulina*) *lacrima* (Reuss); *Pseudopolymorphina incerta* Egger; *Nodosaria proboscidea* Reuss; *Vaginulina* sp. (d'Orbigny); *Dorothia elongata* Finlay. *Ostracoda. Cytheridia punctura* (Schmidt); *Trachylebris communis* (Israelsky); *Cytherella tuberculifera* Alexander; *Cytherreloides swaini* Brown; *Cytherreloides sohni* Brown; *Cytherideis mayeri* (Howe & Garrett).

Correlations were attempted with the Austin, Taylor and Navarro stages of the Texas Upper Cretaceous. The majority of the fossils identified correlated with the Upper Taylor rather than the Navarro as has been previously reported. Two explanations may give light to this obvious incongruity, first, that this area has been wrongly mapped due to generalities assumed during the stratigraphic-megafossil investigations. The second explanation is that the Peedee is considerably older at its base than has been previously supposed. Much more work is needed on the micropaleontology of the Peedee in order to determine its exact correlatives and to place it in its proper position in the Cretaceous time-scale.

Paleoecologic and faunal analyses of some fossil assemblages of the Cretaceous Black Creek and Peedee formations. C. EVERETT BRETT. The Upper Cretaceous formations of the North Carolina Coastal Plain are in ascending order: 1) the Middendorf, 2) the Black Creek dark gray to black shale, and 3) the Peedee gray glauconitic sandstone. L. W. Stephenson in 1923 (North Carolina Geological Survey Bulletin, v. 5) defined the Snow Hill calcareous member of the Black Creek formation for "convenience of reference" as that part of the top of the Black Creek formation which contained fossils. According to modern usage, this is not a valid manner in which to define lithostratigraphic units. A nearly completed study of the Foraminifera, macrofossils and sediments at the base of the Peedee and at the top of the Black Creek formations indicates that a fauna of the same age (*Exogyra ponderosa* zone) as that used to define the Snow Hill member occur in both the Peedee and Black Creek lithologies. Fossil bearing outcrops on the Cape Fear River at milepost 49 and milepost 60 were compared with outcrops along the Contentnea Creek downstream to 7.1 miles below Snow Hill, North Carolina (the type locality for the Snow Hill member), and at Scuffleton, North Carolina. The Foraminifera in all the outcrops studied except those in the top of the outcrop at Scuffleton cor-

related well with the Foraminiferal fauna of the Upper Taylor marl of the Texas Coastal Plain.

Macrofauna were scarce, especially along the Contentnea Creek, but when present they indicated a time horizon in agreement with Foraminiferal data.

Sediments were studied by separating each sample into sand, silt, and clay fractions. The sand fraction was screened to the five standard Wentworth classes and each class was studied under a binocular microscope to determine the volume percent of mineral constituents, Foraminifera, plant fragments, and shells for the whole sample. A comparison of 7 sediment samples taken at the type locality of the Snow Hill member with 9 Peedee and 3 Black Creek samples showed: 1) the Snow Hill lithology appears nowhere along the Cape Fear River (the best exposure of Upper Cretaceous rocks in the Atlantic Coastal Plain), although workers in the past have described certain layers as being the Snow Hill member because of the presence of fauna from the *Exogyra ponderosa* zone and 2) the lithology of the Snow Hill member compares more favorably with the lithology of the Peedee formation than it does with the lithology of the Black Creek formation although Snow Hill sediments are finer grained and darker than typical Peedee rocks.

On the basis of this new information I believe that the term "Snow Hill calcareous member of the Black Creek formation" should be dropped from the literature and that the sediments at Snow Hill, North Carolina be included as part of the Peedee formation as well as other similar unlaminated sediments from other localities. Division of either the Black Creek or Peedee formations into members is not warranted. The term Black Creek formation should connote dark gray to black laminated shales with intercalations of clean sand lying conformably beneath the unlaminated Peedee formation.

Dunite bodies at Holcombe Branch and Democrat, in Madison and Buncombe Counties, N. C. JAMES A. RAY. A report is given on studies made of the mineralogy and geology of two dunites located in Western North Carolina. Both of these dunites are rather small in area, the Holcombe Branch deposit being some 4400 feet in length, and some 1700 in width at its widest point, and the Democrat deposit some 6800 feet in length and 600 feet in width at its widest point. Both are lenticular in outline and are composed essentially of granular olivine-peridotite. Associated minerals thus far identified include chromite, chlorites, serpentines, nickel-silicates, anthophyllite, chalcedony, actinolite, corundum, spinel and cobaltiferous manganese oxides.

A geochemical survey was made on the Holcombe Branch area for nickel, using soil samples. The nickel content of these soils varied from 0 to 2400 ppm, with four sites yielding the higher readings.

Nickel-silicate minerals, together with the cobaltiferous manganese oxides, are found in some abundance in the Democrat deposit and are recommended for further investigation as to the commercial qualities and quantities actually present.

The sediments of the Beaufort Inlet area, N. C. R. WESLEY BATTEN AND ROY L. INGRAM. A total of 202 bottom samples was collected from the Beaufort Inlet area to determine the distribution of sediments and the relation of the sediments to the inlet environment. On the basis of bottom topography and currents, the gorge, channel, and zone of wave action environments were delineated.

The gorge environment is characterized by very rapid currents and a constricted deep water channel. Dredged and naturally maintained channels of relatively deep water and currents of normal velocity of approximately 2 knots mark the boundary of the channel environment. The zone of wave action environment encompasses the major part of the study area. Topographically this environment ranges from strand line to offshore deep water, but is characterized primarily by wave induced currents which affect the nature of the sediments.

Seven types of bottom sediments, predominately sand decreasing in size away from the gorge environment, were recognized on the basis of size range and mode.

Measures of median diameter, one percentile diameter and kurtosis when plotted as variables showed a grouping of sediments into particular environment corresponding to the gorge, channel, and zone of wave action environments.

The comparison of sediments to environment showed that the gorge environment contained the coarsest sands, the highest percentage of shell debris, and was mesokurtic to platykurtic. Closely associated with the gorge, the channel environment sediments exhibited less material of very coarse size, less shell debris, and was mesokurtic to very leptokurtic. The zone of wave

action environment is characterized by finer sediments, generally the lowest carbonate percentages, and variable kurtosis.

Coarse-fraction analysis revealed a relationship between the components of the coarse-fraction and the environments. Gorge sediments were characterized by coarse shell debris and channel sediments were characterized by the presence of aggregates, glauconite, and broken foraminiferal tests. More foraminifera, glauconite, less shell debris, and presence of wood fibers typified the zone of wave action.

Hausdorff transforms of bounded sequences. J. H. WELLS. It has been shown by Barone [Duke Math. J. vol. **5** (1939) pp. 740–752] that the special Hausdorff sequence to sequence transformations of Hölder, Cesaro and Euler have the property that the set of limits points of the transform of each bounded complex number sequence is connected. In this paper we prove the following extension: Let ϕ be a complex valued function of bounded variation on [0, 1] such that $\phi(0+) = \phi(0)$ and $\phi(1) - \phi(0) = 1$ and let $H(\phi)$ be the regular Hausdorff transformation generated by ϕ. The set of limit points of the $H(\phi)$ transform of each bounded complex number sequence is connected if and only if $\phi(1-) = \phi(1)$.

On the canonical form of certain osculants. J. W. LASLEY, JR. This paper was designed to make available in simple form the equations of the conics of 2nd, 3rd, 4th order contact with a curve at a given point of it. Especial emphasis was given to the osculating parabola, the osculating equilateral hyperbola and the osculating conic. The relation of certain of these osculants to Transon's aberrancy was mentioned, as was the possible application to the theory of transition curves.

A metric representation of complex numbers. H. M. NAHIKIAN. In this paper an isomorphism is established between the matrices $\begin{bmatrix} a & b \\ -b & a \end{bmatrix}$, with elements in the real field, and the complex numbers $a + bj$, $j^2 = -1$. The correspondence is established by the identification of matrices $\begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$ with unity and $\begin{bmatrix} 0 & 1 \\ -1 & 0 \end{bmatrix}$ with the imaginary unit j. The determinant $\begin{vmatrix} a & b \\ -b & a \end{vmatrix}$ is identified with the norm $\| a + bj \| = a^2 + b^2$. All of the algebra of complex numbers is developed by means of matrices.

An angle relation associated with two parabolas. L. L. GARNER. Let two parabolas have perpendicular axes, and suppose two parallel tangent lines are drawn, one to each of the parabolas. (These lines may be coincident in the event the parabolas have a common point of tangency.) Suppose, further, that a secant line is drawn through the points of tangency, cutting the parabolas in two other points. If the reference frame is chosen so that the coordinate axes are parallel to the axes of the parabolas, and if μ and m denote the slopes of the parallel tangents and the secant line, respectively, and if θ_1 and θ_2 are the angles formed by the secant line at the other two points of intersection with the parabolas, then

$$\tan\theta_1 \cdot \tan\theta_2 = -\frac{m(m-\mu)^2}{(m^2\mu - m + 2\mu)(2m^2 - m\mu + 1)}.$$

In particular, if the secant line is perpendicular to the parallel tangents, then $\tan\theta_1 \cdot \tan\theta_2 = \frac{1}{4}$. If $m\mu = 1$, then

$$\tan^2 2\alpha \tan\theta_1 \tan\theta_2 + 1 = 0,$$

where $\alpha = \arctan m$.

Effects of heat treatment and mechanical deformation on dark conductivity of AgCl crystals. H. P. LAYER.[4] The structure-sensitive region of dark (ionic) conductivity of AgCl crystals has been studied as a function of thermal history and during plastic deformation. The room temperature conductivity (approximately $10(-8)$/ohm-cm) of an as-received Harshaw crystal was increased 40% by careful annealing in air at 420°C. This annealed value is decreased by a factor of 2.3 if the hot crystal is rapidly cooled (20 minutes). Further annealing increased the conductivity toward its highest value. These results may be understood in terms of a polyvalent negative ion which tends to segregate at dislocations and sub-boundaries. A rapid plastic extension of several percent produces a transient increase in conductivity over and above the relatively permanent effect due to excess dislocations and studied by W. G. Johnston. This pulsed conductivity follows the strain rate pulse even at liquid nitrogen temperatures indicating a relaxation time of no more than 0.02 sec. The effect is pre-

[4] Work supported by the U. S. Atomic Energy Commission and Office of Ordnance Research, U. S. Army.

sumably due to excess interstitial silver ions. Quantitative evaluation of the conductivity increase per unit strain rate, the effect of dislocation content on the pulse magnitude, and extension of the experiment to lower temperatures are in progress.

General nuclear reactor control rod theory. RAYMOND L. MURRAY. The presence of neutron-absorbing control elements in a nuclear reactor creates asymmetries in the neutron density distribution. The density must be expressed in terms of infinite series of products of Bessel and trigonometric functions. Analytic methods have been developed to determine the compatible nuclear fuel content and geometric configuration of the reactor and control absorbers. The size, radial and angular location, and composition of all rods may be different. The procedure consists of solving the three-dimensional (r, θ, z) diffusion equations for two neutron groups (fast and thermal) subject to boundary conditions at the reactor surface and at the surface of each control rod. Transformations of Bessel function series from one coordinate system to another are employed in the application of boundary conditions. *A* condition is found on criticality of a reactor with M eccentric and one central rod in terms of a vanishing determinant of order $M + 1$. The resultant analytical expressions are applicable to reactors in which regulating and control rods of different reactivity values are present, and to stages in the operating cycle when one or more rods of a symmetric array are removed from the core.

Calculation of effects of burnup on reactor properties. SYED AHMED HASNAIN. A method is presented for the calculation of effects of long-term operation on the properties of a reactor, viz. quantities of various fuel isotopes present, conversion ratio, fraction of power contributed by each isotope, flux amplitude and reactivity remaining at any time, from which the core lifetime can be obtained. The model used is a fixed fuel, effectively homogeneous thermal reactor running at constant power. The space dependence of the flux is accounted for by assuming that the bare-equivalent flux shape holds, and that the flux is separable into space and time parts. Extensive use is made of the burnup functions,

$$B_l(x) = \frac{1}{V} \int \phi^l e^{-\phi x} \, dV$$

Transient heat transfer in reactor coolant channels with thin fuel elements. E. L. ARNOLD AND W. O. DOGGETT. Two heat transfer models are considered to determine the change in coolant temperature spatial distribution as a function of time. The first model assumes instantaneous heat flow to the coolant. The second considers the thermal time delay in transporting heat to the coolant. The initial conditions for both models are chosen as the steady-state conditions for a chopped-sine power distribution.

General solutions are obtained for each model using both steady-state operation and exponentially rising power. Curves are plotted for specific reactor parameters based on both models. A comparison is made of the two models in order to determine where the assumption of instantaneous heat flow is applicable. The results show that this assumption is applicable for long reactor periods and is in error only a few per cent for periods equal to the characteristic heat and coolant transfer times.

A time correlation technique for investigating three successive nuclear transformations. W. O. DOGGETT AND T. E. TODD. A new technique is proposed for determining the number of true prompt triple coincidences in a time interval that arise from a radioactive decay cascade. The method requires the detection of the three types of radioactivity and subsequent determination of a correlation function. It is shown that the conventional triple coincidence counting technique is a special case of this correlation method and that the correlation method is free in principle from counting rate limitations that restrict the range of usefulness of the coincidence technique.

The dispersion and relative error of the correlation measurement are determined. The technique is extended to the cases of delayed emission of the second and third members of the cascade. Additional correlation functions are developed for the measurement of the half lives of the emitters of the second and third types of radiation. This technique should be very useful in the measurement of millisecond half-lives.

The genitalia of Elateridae (click beetles). WALTER M. KULASH. The Elateridae comprise a large family of beetles popularly referred to as click beetles or snapping jacks, because of their habit of snapping upright when placed on their backs. There are over 1100 species in the U. S. and Canada and about 200 species in North Carolina.

Many of these are of economic importance because of the damage they do to corn, tobacco, potatoes, and other crops.

The identification of many closely related species is difficult because of the lack of clear external morphological characters. The male and female genitalia have been used to study the phyletic relationships and to differentiate species. The study of the internal genitalia involves certain techniques in the killing and preparation of specimens for dissection. In some of the larger specimens, the male genitalia may be pulled after relaxing the specimens in boiling water, to which a little detergent has been added. Large specimens, or very dry specimens, require a longer period of boiling, and carefully timed clearing in hot or cold potassium hydroxide, 10%.

In the examination of the female genitalia, the abdomen is first removed from the specimen, soaked in water or cleared in potassium hydroxide. Slide mounts of male and female genitalia may be made in Hoyer's solution. If it is desirable to have unmounted genitalia available for future study, they may be placed in a micro-vial of glycerine.

Our current study of elaterid genitalia is confined to the genera *Melanotus* and *Glyphonyx*, expecially the latter genus. The most apparent differences in the male genitalia of both genera are in the shape of the lateral pieces. In *Glyphonyx* females, differences appear in the size and shape of the chitinized plates of the bursa copulatrix, the size and number of the teeth on these plates, and in the shape and structure of the base of the spermathecal duct.

Hieroglyph, or gravity aligned steering substance in bee dance? A. L. PICKENS. Following the remarkable work of Dr. von Frisch in studying bee communication some writers have been inclined to attribute to the bee an intelligence equal to that of man when he first began to write in hieroglyphs. Study of physical reaction of fluids in a container subjected to motions corresponding to those performed by the bee in its direction-imparting dance, tend to show that back of what some have taken for an ideograph may lie a comparatively simple physical explanation, and the supposed ideograph may not be an extraordinarily great saltation.

Observations on the spawning and early development of the common scallop, Pecten irradians. L. M. OUTTEN. During the summer of 1956, *Pecten irradians*, the common scallop, was used for observations of spawning and early development at Marine Biological Laboratory, Woods Hole, Massachusetts, under the guidance of Dr. R. D. Allen. Individuals stored on a sea water table at 18° to 18.5°C. were placed separately in small bowls and covered with sea water. In the first part of the study, the water was gradually warmed by an electric lamp to about 26°C. With an additional lamp, the temperature of the water was increased to 30°C. and higher. There was little response to the change of temperature. In later studies, heated sea water was added to the bowls containing the specimens raising the temperature to 32°C. By adding chipped ice, the water in the bowls was cooled to 28°C. or lower. As the temperature of the water was reduced, the release of eggs was observed seventeen times at temperatures from 25° to 32°C., twelve times of which were at temperatures from 25° to 28.1°C. On thirteen occasions, sperm was noted to be given off, eleven times at water temperatures from 23° to 28°C. The pinkish eggs were given off in a fine spray or small chunk-like masses, generally accompanied by movements of the valves. Sperm discharge was usually in a fine mist, at times like little puffs of smoke, and often without any movement of the shell.

Considering the effects of light on spawning, there were four instances of egg release and three of sperm discharge in well-lighted conditions and only a small number of eggs given off by one specimen in a darkened situation.

The general form and structure of the ovum and the developmental stages to the late veliger (five days old) were observed.

Differentiation and proliferation in the development of amphibian adrenals. LAURA G. ANDERTON. Literature on the development of the adrenal glands contains many direct and indirect implications that an inductive sequence might exist and account for the morphological relationship between the two adrenal components with different germ layer derivation. To investigate this possibility in amphibia, a study was made of the developmental chronology, differentiation, and proliferation of adrenal components under the following conditions; normal development and extirpation of trunk neural crest (*Amblystoma punctatum*); conditions of physiological stress involving critical temperature change (*Rana pipiens*) and injection of ACTH (*Triturus viridescens*). It was concluded that the

fuchsinophil cells are both the embryological and secretion cycle precursors of the osmophil cells. It was demonstrated that differentiation of both components can take place without contact of the adrenal components. In animals lacking trunk neural crest there was a marked reduction in adrenal cortical cells in early larval life but secondary proliferation occurred at an increasing rate thereafter. This proliferation took place without contact of the adrenal components and in late larval life occurred in the absence of all but a relatively small percentage of the normal adrenal medullary cell complement. Evidence is presented which shows that an inductive sequence involving contact of the adrenal components is not necessary for their differentiation and proliferation. The importance of the vena cava in the morphogenesis of amphibian adrenals was demonstrated both in normal and in experimental animals.

The effects of age on hyperplasia in the remaining rat kidney following unilateral nephrectomy. CHARLES E. MCCREIGHT. Unilateral nephrectomy in young adult rodents has been shown to produce enlargement of the remaining kidney accompanied by specific cytologic changes, including increased mitotic activity (Sulkin, '49; Rollason, '49; McCreight, '58). In the present study the response of the remaining kidney in young and senile rats is compared.

Six young adult albino rats, 120 days of age, and 4 senile animals, 1160 days of age, underwent removal of the left kidney. These kidneys were fixed in formalin to serve as control organs. Three days after nephrectomy the animals were sacrificed, and the remaining kidneys were removed and fixed. Paraffin sections of all the kidneys were cut at 10 microns and stained with the Feulgen nuclear stain and fast green. Percentages of cells in mitosis in the proximal convoluted tubules of both control and remaining kidneys of each age group were determined.

Mitotic figures are very rare in the control kidneys of both young and old rats. In the young animals the response of the remaining kidney following the operative procedure includes a considerable increase in per cent of cells of the proximal convoluted tubules undergoing division. The increase in mitotic activity of these cells in the remaining kidneys of the senile animals is not pronounced.

The effects of temperature and salinity on the development of Axiothella mucosa (*Andrews*). MURAD-B. M. MOHAMMAD AND C. G. BOOKHOUT. The effects of variations in temperature and salinity, apt to be encountered in nature, on the development of *Axiothella mucosa* (Andrews) in the laboratory have been studied from the fertilized egg to the eleventh day of juvenile life. Eggs, larvae and juveniles which have been held in the optimum temperature (25° to 30°C.) die within 24 hours when transferred to a temperature of 35°C. A temperature of 15°C. appeared to be lethal to the eggs. The larvae and the juveniles, however, are more resistant to low temperatures.

A salinity of 16 parts per thousand proved to be lethal to the eggs. Larvae and juveniles which have been reared in the optimum salinity (32 to 40 p.p.t.) show swellings on transfer to a concentration of 16 p.p.t. and readjust to the dilute medium after approximately one week. With respect to rate of development, the low temperature acts as the low salinity in retardation of larval and juvenile growth. It was observed, however, that the temperature and salinity of the sea water exert their influences on the development of the Maldanid independently, that is to say: the salinity changes undergone do not affect temperature tolerance; and vice versa.

The use of radioactive gold (Au^{198}) to trace movements and field activity in Eastern harvest mice, Reithrodontomys humulis humulis (*Aud. and Bach.*) STEPHEN V. KAYE. Four Eastern harvest mice were tagged with subcutaneously inserted 20 gauge Au^{198} wires measuring 10 mm. in length and varying in activity from 0.7 to 4.5 mc. Application of the wires was achieved by pushing them through a hypodermic needle inserted under the animal's skin in the abdominal region. Tags inserted by this method had no apparent effect on the behavior of the animals, nor were any of the radioactive wires lost. Tagged harvest mice were located in the field with a portable Geiger counter at various intervals over a period of 6 to 10 days. At the end of each trial the mouse was retrieved and sacrificed. Two mice were tagged at different times during the summer of 1958; one mouse was tagged in the winter of 1958–59, and a fourth animal was tagged in the spring of 1959. During all three seasons of the year, tagged mice lived in small globular grass nests constructed under vegetation, but built on the surface of the ground. Twelve such nests were used by the tagged mice; two mice had three nests, one had four nests,

and one had two nests. Daily use of these nests was rotated, and all nests appeared to be situated on the periphery of each animal's range. Harvest mice stayed in the nests during the daylight hours, but moved freely around the field at night. This investigation was supported by a research grant from the North Carolina Academy of Science.

Expanding student horizons through science roundtables. JAMES W. MANLEY. Science Roundtables held at the Charlotte Children's Nature Museum appear to be an effective method for demonstrating to above average teenage students in Charlotte and Mecklenburg County the interdependence of the various scientific disciplines and the stabilizing role, in any culture, of the Humanities.

The Roundtable program was begun early in 1958. To date 23 programs have been presented by 19 different scientists, educators and other professional people to approximately 150 students selected by science teachers. The most recent Junior High series was attended by 44 students from 13 different schools; the Senior High series was attended by 33 students from 10 different schools.

The following topics suggest the scope of the program: "Your Atomic Future"; "The Chemistry of Life"; "Psychiatry—What's it all About?"; "Medical Research—Allergies"; "Is the Scientist Always Right?"; "Cosmology and the Nature of the Universe"; "Paleontology—Man in the Evolution of Life"; "The History of Man—The Archeologist Studies Egypt"; "Inside Matter through Optics"; "Genetics and Radiation"; and "Mathematics—a Philosophy or Science".

Perhaps the greatest significance of this program is that it suggests a workable method for tapping, economically, a huge resource of community manpower in order to present effective programs of high quality to the above average student.

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

1958–1959

546TH MEETING, OCTOBER 14, 1958

W. S. POLLITZER (Anatomy): *Use of Gene Frequencies in Physical Anthropology: Gullah Negroes of South Carolina.* The degree of racial affinity between populations has long rested upon certain morphologic characteristics, such as skin color, hair form, or nasal width. The blood types, determined by genes, provide a new method of analysis, as their frequency varies with different races.

The Gullah Negroes around Charleston, S. C., were studied for blood factors and morphologic traits. Findings were compared with similar data for West African Negroes, American Negroes, and Whites. Genetic and morphologic methods of analysis showed close agreement. By both, the Gullah Negroes were more similar to the African and further removed from Whites than the average American Negro. A lower degree of hybridization is the most likely cause of their distinct biological position.

D. GORDON SHARP (Biophysics): *Electronmicrographic Study of Viruses.*

547TH MEETING, NOVEMBER 11, 1959

M. R. CARRIKER (Zoology): *A Glimpse of the XVth International Zoological Congress and Some British Marine Laboratories.*

W. F. WHITE (Geology): *Geomorphic History of Lake Tsala Apopka and the Withlacoochee River, Fla.*[1] The Withlacoochee and Hillsborough Rivers have common headwaters but are diffluent near Richland in southeastern Pasco County. From this point of diffluence the Withlacoochee flows northwestward 60 miles to Dunnellon, where is turns westward, crosses the Brooksville Ridge on a bedrock shoal, and debouches into the Gulf of Mexico at the distal end of a promontory. Between the point of diffluence and the rock shoal at Dunnellon it follows a broad, flat-floored valley which includes the large lake, Tsala Apopka. The river's relation to the lake is peculiar. It merges with the upstream end of the lake; then dissociates itself from the lake and flows around three sides of it. The lake at its lower end drains back into the river.

[1] With permission of the Florida State Geological Survey.

It is suggested that most of the Withlacoochee Valley between Richland and Dunnellon is the bed of a former large lake which drained southward via the Hillsborough River into Tampa Bay. Solution of carbonate rock in the valley wall opened a new outlet near the head of this ancestral lake at Dunnellon and the drainage direction between Richland and Dunnellon was reversed. Lakes Tsala Apopka and Panassoffkee and many smaller lakes and swamps seem to occupy low places in the floor of the former lake.

The route of the Withlacoochee below Dunnellon is shown to be new by a bore hold in its channel upstream from Dunnellon which penetrated channel fill to a depth 100 feet below the level of the Dunnellon bedrock shoal. Also the Pamlico marine terrace crosses the valley of the Withlacoochee below Dunnellon abruptly without turning upstream along the valley walls. This suggests that the valley was cut after the terrace had been abandoned by the sea. But the terrace extends a long distance up the Hillsborough Valley attesting the presence of an estuary there at Pamlico sea level. The newness of the lower Withlacoochee Valley is also suggested by its debouchure at the seaward end of a promontory. Conversely, the Antiquity of the Hillsborough Valley is suggested by its debouchure into Tampa Bay, the largest estuary of peninsular Florida.

548TH MEETING, DECEMBER 9, 1958

ALFRED T. BRAUER (Mathematics): *Computation of the Greatest Root of a Positive Matrix.*

Discussion of Society's Activities and Functions. President Ingram read comments from several solicited letters relative to time, place, frequency of meetings and the nature and quality of programs. The difficulty of arranging programs by the Program Committee Chairman was discussed at some length. It was voted to continue the meetings at Philips Hall. A count of a letter poll on time of meeting showed 34 for 7:30 p.m. and 20 for 8:00 p.m. It was suggested that the Program Chairman ask several key men on the campus to assist him in inviting individuals to present suitable papers to the society.

549TH MEETING, JANUARY 15, 1959

GEORGE L. PENICK (Pathology): *The Pathogenesis of Some Acquired Hemorrhagic Disorders.*

550TH MEETING, FEBRUARY 10, 1959

GEORGE COCOLAS (Pharmacy): *A New Application of the Michael Addition.* The base catalyzed Michael addition between diethyl acetamidomalonate and ethyl acrylate or ethyl crotonate may give one of two products, depending on the reaction conditions. Mild conditions, i.e. room temperature, yield the corresponding normal addition product, diethyl N-acetyl-α-carbethoxyglutamate, whereas, more vigorous conditions such as the reflux temperature of ethanol favor the formation of a 5,5-dicarbethoxy-2-pyrrolidinone. The formation of the pyrrolidinone is the result of a concomitant normal Michael addition and an intramolecular cyclization.

The hypothesis of the cyclization is not unlikely for two reasons, namely: the elevated temperatures employed for the cyclization favor the bimoleculary hydrolysis of amides, and the favored "crescent-like" configuration assumed by the molecule which places the acetylamino group in proximity of a carbethoxy group. The combination of these two situations affords a plausible explanation of the synchronous normal Michael addition and cyclization.

Degradation studies of the 5,5 dicarbethoxy-2-pyrrolidinones formed from ethyl acrylate and ethyl crotonate to the corresponding dicarboxylic and monocarboxylic acids were presented as evidence for the proposed cyclic structure.

It is also possible to affect the formation of an "abnormal" Michael addition which on subsequent cyclization will form a corresponding 3,5-dicarbethoxy-2-pyrrolidinone. Such a rearrangement can be induced by employing equivalent amounts of base in order to stabilize the acrylic ester-acetamidomalonyl carbanion adduct, the first intermediate formed in a normal Michael addition, in order to promote the formation of a succeeding cylobutanone intermediate. The cyclobutanone intermediate on subsequent cleavage and bimolecular amide alcoholysis will give the 3,5-dicarbethoxy-2-pyrrolidinone.

Steps are presently being taken for an unequivocal synthesis of 3,5-dicarbethoxy-2-pyrrolidinones.

CLAUDE PIANTADOSI (Pharmacy): *Studies on Basic Plasmalogens.*

551ST MEETING, MARCH 10, 1959

EUGEN MERZBACHER (Physics): *Symmetry in physics.* The recent overthrow of parity in the weak interactions has led to a careful re-examination of the various laws of symmetry which have been accepted uncritically. We have no a priori knowledge how such quantities as charge, particle, magnetic moment transform under spatial reflection. Ampere's experiments with magnets and currents suggest that electromagnetic phenomena are invariant under reflection if the mirror image of a north pole is a south pole, and vice versa. This experimental fact led Ampere to an understanding of magnetism in terms of elementary currents. The recent parity experiments suggest that the mirror image of, say, Cobalt is anti-Cobalt. An experimental test of this assumption is possible if the behavior of a beta-decaying system under the time-reversal transformation is studied. If weak interactions are found to be invariant under time-reversal, one should ask next why strong interactions are separately invariant under the traditional parity operation and under charge conjugation. A simple table-top demonstration of the parity situation in electromagnetism was presented.

552ND MEETING, APRIL 14, 1959

E. T. Browne in Memoriam

J. W. LASLEY. *E. T. Browne as a Person.*

A. T. BRAUER. *E. T. Browne as a Mathematician.*

J. L. GODFREY (Dean of Faculty): *Some Observations on Science and the University of North Carolina.*

553RD MEETING, MAY 12, 1959

I. FOWLER AND W. M. CLARKE (Anatomy): *The inhibition of lens-inducing capacity of the optic vesicle with adult lens antisera.* An antiserum (rabbit) to adult chicken lens which gave a homologous precipitin reaction with an antigen dilution containing 0.16 g. of nitrogen per ml. was prepared. Serum agar diffusion patterns indicated the antigen-antiserum system contained at least five components. Optic vesicles from chick embryos of 4 to 7 somites were dissected free of ectoderm and cultured 18 hours in medium containing 1:20 dilution of antiserum globulin. The vesicles were then enclosed in competent ectoderm and cultured 3 days in a medium free of antiserum. Induction of lens occurred in 2 of 61 cases (3%). Induction of lens resulted in 35 of 66

cases (53%) in the control vesicles cultured in a 1:20 dilution of normal rabbit serum and subsequently enclosed in ectoderm. The antiserum did not prevent formation of otocyst under similar conditions.

Chick embryos of 7 somites stained with antilens serum conjugated with fluorescein isocyanate (Coon's technique) revealed antigens to which antiserum was directed were localized in the optic vesicle and brain but not in lens ectoderm. In embryos of 19 somites and older localization was evident in both optic vesicles and lens ectoderm.

The observations suggest the presence of a lens-like antigen in the optic vesicle prior to lens induction.

ANCEL MEWBORN (Mathematics): *Generalizations of Some Theorems on Positive Matrices to Completely Linear Operations in a Normal Linear Space.* Dr. Mewborn's presentation was based on a doctoral thesis which was adjudged the winner of the Coker Award. The committee on the Coker Award was comprised of J. W. Straley, chairman, C. R. Bell, and G. O. Doak. At the conclusion of the meeting, Mr. Mewborn was presented with a check for $50.00.

The following business was also transacted:

546th meeting. Fifty-six associate members were elected. Fifteen full members were nominated and elected: F. E. Bell (Biochemistry), Ann F. Blevins (Botany), C. V. Briscoe (Physics), G. H. Cocolas (Pharmacy), W. C. Denison (Botany), W. W. Forrest (Pathology), Paul Hubbard (Physics), R. A. King (Psychology), Ralph Penniall (Biochemistry), Felix Pirani (Physics), N. F. Rodman (Pathology), R. H. Sager (Dentistry), D. G. Sharp (Bacteriology), G. H. Spooner (Biochemistry), J. M. Wells (Mathematics).

547th meeting. Two associate members were elected. Three active members were nominated and elected: George R. Cannefax (Public Health), T. C. Washburn (Public Health), M. J. Widra (Public Health).

553rd meeting. The auditing committee, consisting of H. G. McCurdy (Chairman) and J. S. MacNerney, reported the books of the Secretary-Treasurer to be in order.

The report of the Secretary-Treasurer was presented and received. Included was an announcement that the North Carolina Academy of Science was to contribute $1.00 per member to the journal instead of $.50 plus any unexpended balance in the Academy Research Fund for the year (1958–1959) to the society.

The Nominating Committee (C. W. Hooker, W. L. Engels, and J. C. Morrow) presented the following nominees for 1959–60:

President—J. L. Irvin

Vice President—A. V. Masket

Secretary-Treasurer—M. R. Carriker

All reports were duly received and the nominees were unanimously elected.

The following changes and ammendments to the constitution were presented by President Ingram.

On page 5 before the section on duties of the Editorial Board insert: Program Committee. The Program Committee shall consist of the Vice President as chairman and two other members appointed by the President.

On page 4 under duties of the Vice President add: "With the aid of the Program Committee" . . . he shall arrange the programs for the society.

The amendments were duly received or accepted and voted as a part of the constitution.

HERMAN O. THOMPSON, *Sec.-Treas.*

ABSTRACTS OF PAPERS PRESENTED AT THE SPRING MEETING OF THE SOCIETY OF NORTH CAROLINA BACTERIOLOGISTS AT DURHAM, N. C. ON APRIL 4, 1959

1. The effect of diet on disease in the neonatal pig. *James G. Lecce and Gennard Matrone, Department of Animal Industry, North Carolina State College, Raleigh.* Neonatal serum protein patterns from unfed pigs were compared with serum from mature pigs. This yielded a profile of an immature serum protein pattern and a mature pattern. The capacity of 4 different diets to augment and influence changes from the immature to the mature serum protein pattern was determined. It was found with pigs nursing the sow that not only were there rather intense and immediate changes in serum proteins, but there was also a continuous and rapid alteration toward the mature serum protein pattern. Pigs fed an amino acid milk had little ability to promote normal changes associated with this serum protein maturation. Pigs fed cow's milk and artificial milk were intermediate between the two extremes of sow's milk and amino acid milk. That is, in the first 4 to 7 days, little or no changes were observed in the serum protein patterns; however, from then on, these pigs started milk changes observed with the sow fed pigs, except at a slower rate. Until these serum protein changes occurred, the piglet gained little weight and was extremely susceptible to diarrhea, bacteremia and death. The sow, then, with her colostrum and milk, rapidly fills in the deficiencies of the new born, sparing the piglets a difficult and stressing adaptation to an extra-uterine environment.

2. Volutin: Structure, function, formation. *Abe Widra, Department of Bacteriology and Immunology, University of North Carolina Medical School, Chapel Hill.* Volutin is re-examined as a cytological entity and a working hypothesis is presented on the mechanism of formation of this substance. The cardinal point of the present investigation shows lipids to be a major component of the volutin granule. These lipids are loosely bound to or overlaid with protein. Other components include ribonucleic acid, probably linked to the protein, and energy-rich polyphosphates. These polyphosphates may be complexed with the ribonucleic acid through a divalent cation such as magnesium.

The various functions ascribed to volutin are reviewed.

The simultaneous occurrence of lipid and high-energy phosphate in a discrete well-organized granule which apparently arises de novo indicates a common precursor substance. Under anaerobic conditions, or after phosphate starvation and subsequent feeding, it is hypothesized that 2-phosphoenolpyruvate furnishes both high-energy phosphate and, after decarboxylation, "active acetate" for lipid synthesis. Pyrophosphate, split out during lipid synthesis, acts as "primer" in the reaction: ATP + phosphate primer $\rightleftharpoons$ ADP + polyphosphate. Phosphoenolpyruvate may accumulate during a time of nutrient imbalance when the rate of phosphate cycling from 1,3-diphosphoglyceric acid to ADP exceeds the rate from phosphoenolpyruvate to ADP and all newly formed ATP is channeled toward esterification of hexose. Addition of ADP would therefore restore balance and inhibit volutin formation.

3. Studies on the formation and activity of arginine desimidase and citrullinase of a lactobacillus species. *William R. Bibb and W. R. Straughn, Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill.* It has been reported for bacteria possessing both arginine desimidase and citrullinase that citrullinase activity with citrulline as the substrate is demonstrable with cell-free preparations but not to any appreciable extent with whole cells. The usual explanation is that of cell impermeability to citrulline. The results of the present investigation are interpreted as indicating a specific permease system for citrulline which can be induced by the intracellular formation of citrulline from arginine or from extracellular citrulline. With cell suspensions of a heterofermentative lactobacillus species grown in a casein hydrolysate-yeast extract-glucose medium, both arginine desimidase and citrullinase activities were demonstrated when arginine was the substrate. No appreciable activity within 60 minutes was obtained when citrulline was the substrate. With sonically disrupted cells citrullinase activity was equally

demonstrated using either arginine or citrulline as the substrate. When arginine was added to the growth medium both arginine desimidase and citrullinase activities of whole cells were greatly increased using arginine as the substrate. With citrulline as the substrate, citrullinase activity also was very appreciable, indicating that the arginine-grown cells were permeable to citrulline as well as to arginine. When citrulline was incorporated in the growth medium, cells with increased citrullinase activity also were obtained. Specific enzyme actions were verified by chromatographic determinations of arginine, citrulline and ornithine. Ammonia was determined by Nesslerization. Carbon dioxide was determined manometrically using acetate buffer at pH 5.8 with sodium arsenate but no phosphate in the system.

4. The morphology of tubercle bacilli grown in the presence of basic polypeptides and histone. *J. K. Spitznagel, Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill.* Spitznagel and Sharp have shown that magnesium and sulfate deficiencies operating singly or together in simple chemically defined media induce the appearance of populations consisting principally of long forms in cultures of *M. bovis* and *M. tuberculosis*. Two basic polypeptides derived from calf thymus and calf thymus histone have been found to induce similar long forms in such cultures. The length of these forms may exceed 14 microns and their breadth is usually .5 microns. The internal structure is altered by the appearance of numerous granules, vacuoles and rings. These structures may be seen by phase microscopy, in stained preparations and by electron microscopy. The long form inducing properties of the basic polypeptides and histones appear to be fully antagonized by magnesium sulfate. These findings are consistent with the notion that the long, barred, vacuolated acid fast bacilli found in certain tuberculous tissues may be displaying the effects of magnesium sulfate deficiency induced by histone like substances.

5. Studies on the nucleotide spectra of microorganisms. *E. J. Smith and R. W. Wheat, Department of Biochemistry, Duke University Medical Center, Durham.* A concave gradient, using ammonium formate at pH 3, was utilized in establishing the elution pattern of a mixture of known nucleotides from Dowex-1-anion exchange resin in the formate form. A profile graph of 260 mu absorbing material eluted indicated that essentially three areas were observed. Cytidine-, adenine-, uridine-, and guanosine-monophosphates appeared early and in that order, while the diphosphates were intermediate and the nucleoside triphosphates were eluted terminally. This sequential position was used as a guide in qualitatively establishing the identity of components in microbial extracts. Using this system, a 10% trichloroacetic acid extract of *Neurospora crassa* revealed, in the following order, the presence of CDP-choline, DPN, AMP, UMP, UDP-N-acetyl-glucosamine, UDP-glucose, ATP, and GTP. The spectrum of a *Bacillus* species appeared to contain in addition to the usual nucleotides, a hitherto unreported guanosinediphospho-uronic acid. Further studies are in progress.

6. Studies on primary actinomycosis of the eye. *Leo Pine, Department of Microbiology, Duke University School of Medicine, Durham.*

7. Fluorescent antibody studies of *Sporotrichum schenckii*. *Ernest W. Chick, VA Hospital, Durham.*

The Elisha Mitchell Scientific Society dedicated its meeting in April to the memory of E. T. Browne, its president during 1932-33, its corresponding secretary since 1951, a member of its editorial committee, and a member of its advisory committee.

The following addresses were given:

E. T. BROWNE AS A PERSON

BY JOHN W. LASLEY, JR.

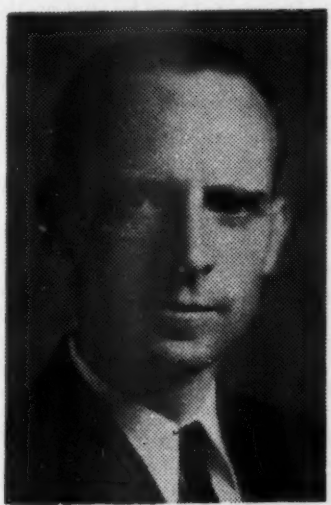
I know of no one about whom nicer things could be and should be said than about Ed Browne. I know of no one less fitted to say them as they should be said than your speaker. And yet, I feel obliged to make the try since I have known him longer and (except for his wife) better than anyone here; in fact, our lives have been very closely entwined for nearly four decades.

My first meeting with Ed took place in the mathematics classroom of E. H. Moore at the University of Chicago in the fall of 1919. After class Mr. Moore, as was his custom, had us introduce ourselves. When Ed found out that I was from North Carolina and I found out that he was from Virginia, we went for each other like iron filings to a magnet. There began a friendship which the years served only to strengthen. Our days at Chicago consisted mostly of intensive study with occasional trips to the Loop for relaxation. Ed proved to be an interesting and agreeable companion. His quick wit and ready repartee, his spritely good cheer, endeared him to everyone. Whether we were on the courts, skating on the Midway, or dining at a 63rd Street restaurant, having Ed along ensured an enjoyable occasion. His speech was a dead give-away of his Southern extraction. Students used to gather around us just to hear him talk.

Ed was the son of a Methodist preacher. He used to say, in his jocular way, that he and his father knew all about the Bible. When harried by a question beyond his depth, he would parry with the remark that the answer to that question came in the part that his father knew.

Once at a meeting of the North Carolina Academy of Science he was addressed by another Academy member as Mr. C., a colleague whom he did somewhat resemble. With no perceptible hesitation Ed replied, "I'm Ed Browne, not Mr. C.; I've just been sick, that's all."

The main facts and dates of his life are to be found in Who's Who in America. He was born November 22, 1894, at Lilian, Northumberland County, Virginia. His early education included schools at Front Royal and Scottsville. His undergraduate college training he received at the University of Virginia from 1911 to 1915. His Master's degree was received from Virginia in 1917; his Ph.D. degree from Chicago in 1926. Before coming to Carolina in 1922, he taught at the College of William and Mary and at Trinity College, Hartford, Connecticut. At this University he rose quickly from assistant to associate to full professor. He served his country in two capacities: as an officer in the Coast Artillery Corps U.S.A., 1917–1918; also as an instructor in the American Army University at Shrivenham, England, 1945.



Dr. E. T. Browne

It was but natural that many honors should be his. He was a member of the Raven Society and of Phi Beta Kappa at Virginia, and of Sigma Xi at Chicago. He was a member of the American Mathematical Society, of the Mathematical Association of America (Board of Governors, 1944–45), the North Carolina Academy of Science (co-founder of its Mathematics Section), the Elisha Mitchell Scientific Society. He was president (1938-39) of the local chapter of Sigma Xi. He served on many of the University's important committees: the Retirement Annuities Committee, the Executive Committee, the Administrative Board of the Graduate School.

He was a coordinator of the local Pre-meteorological Unit in 1943. With these honors went attendant responsibilities. They were met with the same sincerity of purpose and careful attention to duty that were evident from the early fellowships at Virginia and Chicago to the final days of his academic career. Fitting recognition of his achievements and integrity was given by his alma mater just a few years before his death. In an effort to recognize her most distinguished sons, Virginia held a widely-publicized banquet to which were invited those whom she most desired to honor. Ed Browne's name was on the list.

As a teacher he held a high standard of excellence. He expected from his students the same energetic drive toward competence which he believed and practiced. In spite of this, perhaps in part because of it, there was mutual respect and in many cases genuine affection between Ed and his students, some of whom have become nationally known mathematicians. He was a good teacher, clear and inspirational. We regarded him as one of our best.

As a husband and father he was devoted, kind, and understanding. He was faithful to his church, serving for ten years as the superintendent of its Sunday School. He reflected in his daily life those spiritual qualities which were the inner resources that enabled him to meet with such fortitude the physical strains of his latter years.

The first inkling that I had of the malady which finally struck him down came as I was sitting in our joint office one day about fifteen years ago when I heard him coming up the hall tapping his feet on the floor. He said that he first noticed his condition coming on by a peculiar feeling in one of his fingers as he struck the typewriter keys. This was before his overseas service at Shrivenham, although the condition was not far advanced at that time. Upon his return he grew gradually worse. Everything possible was done to remove these physical difficulties that beset him. Hospitals and private clinics here, in Durham, and in New York examined him and gave him treatments. Only a few days before his death a new treatment based upon atomic radiation was being investigated. Through these many years of having to endure a progressive deterioration in his body—a body once so vibrant and alert—there was never evidence of complaint, never abandonment of hope.

Robert Browning put it this way, "I was ever a fighter, so one last fight the best and the last." This last fight came on the last day of March in Memorial Hospital. Pneumonia took over in a body so wracked and weakened by many years of fighting that even so stout a heart as his could fight no more.

His going leaves us saddened; indeed, it leaves us poorer. The University loses a splendid combination of teacher and creative scholar. Many of us have lost not only a colleague, but a close friend. To his family, whose loss is greatest, go out our prayers and sympathy. We have, though, that which cannot be taken from us—a life such as his spent among us. I know of no more fitting characterization of that life than that given by Marc Antony of Brutus on the field at Phillipi: "His life was gentle and the elements so mixed in him that nature might stand up and say to all the world, 'This was a man.'"

E. T. BROWNE AS A MATHEMATICIAN

By Alfred T. Brauer

In general, it is not a great event in the history of a university when a young assistant professor is added to its staff. But it became a milestone in the history of our university, when in 1922 Edward Tankard Browne was appointed as assistant professor on the recommendation of Dr. Lasley. For the first time a mathematician came to this university who was interested in algebra while geometry and analysis were already well represented. From now on, nearly 50 years after the foundation of the graduate school, it became appropriate to speak of a graduate program in mathematics, while 20 years earlier not even enough courses were offered to justify a B.S. in mathematics.

Browne wrote his Ph.D. dissertation under the direction of L. E. Dickson and received the Ph.D. from the University of Chicago in 1926. The topic of his dissertation, "Involutions that belong to a linear class," [4][1] was a problem in algebraic geometry, but uses strongly the theory of matrices. The dissertation was later republished in a slightly changed form [5] and presented as a paper at the annual meeting of the American Mathematical Society in 1927 at Nashville, Tennessee, in the section for geometry. A second paper of Browne's [6] was read by title. It may be remarked that at this meeting 70 papers were presented. Besides the two papers

[1] Numbers in brackets refer to the list of the publications at the end of this paper.

of Browne's, four other papers were given by mathematicians who are now members of our department.[2] I doubt that this high percentage will ever be reached again by us in the future at an annual meeting of the A.M.S.

Browne's second paper presented at this meeting was a paper in the theory of matrices. This theory became his field of greatest interest. In it he published 8 important papers [6, 7, 8, 9, 14, 15, 20, 21], his lecture notes, and his book [3]. Four more papers [11, 16, 18, 22] are more or less connected with his dissertation. They deal with geometrical problems, but all of them use matric theory. They can be better classified as application of matrices to geometry. One number theoretical paper exists [13], but also in it a determinant and its cofactors play an important role. Browne published two elementary books, Introductory Mathematics with Dr. Lasley [1] and College Algebra with Dr. Cameron [2]. In both of them determinants are more thoroughly treated than in similar textbooks. Only in four short notes in the Mathematics Teacher [10, 12, 17] and in the High School Journal [19] are no determinants or matrices used.

Let us now consider some of Browne's research a little more exactly. Dickson in his book, *Introduction to the Theory of Numbers*,[3] solves the following problem: Let a, b, c, e, be integers with $e \neq 0$ and $b^2 - 4ac$ not the square of a rational number. Assume that ξ, η, ζ are a rational solution of $ax^2 + bxy + cy^2 = ez^2$. Then all rational solutions can be obtained as functions of ξ, η, ζ and 3 parameters u, v, and ρ where u and v are relatively prime integers and ρ is rational. Moreover, Dickson gives a method to determine which restrictions must be placed on the parameter u, v, and ρ in order to obtain only integral solutions and all integral solutions. Browne, in a short note [13], gives a much simpler proof for this result than Dickson.

In 1902 the first bounds for the absolute value and for the real and imaginary parts of the characteristic roots of arbitrary matrices were obtained by Bendixson[4] and by Hirsch,[5] and in 1906 Bromwich[6] proved the following theorem. Let $\alpha + i\beta$ be a characteristic root of the matrix A, let $\rho_1 \leqq \rho_2 \leqq \cdots \leqq \rho_n$ be the characteristic roots of the Hermitian matrix $\frac{1}{2}(A + \bar{A}')$ and $\sigma_1 \leqq \sigma_2 \leqq \cdots \leqq \sigma_n$ the roots of the Hermitian matrix $\frac{1}{2i}(A - \bar{A}')$, then $\rho_1 \leqq \alpha \leqq \rho_n$ and $|\beta| \leqq \max |\sigma_\nu|$. In his first paper on matrices [6] Browne obtained a similar result. The matrix $A\bar{A}'$ is a positive definite Hermitian matrix. Let g be its smallest and G its greatest root, hence $g > 0$. Then Browne proved that all roots of A lie in the circular ring $g \leqq |z| \leqq G$. This theorem gives often much better results than the theorem of Bromwich. In particular, if A is orthogonal or unitary, then $A\bar{A}'$ is the unit matrix, hence we obtain a simple proof for the important result that all characteristic roots of a unitary matrix lie on the circumference of the unit circle.

Two years later, in another paper, Browne [9] succeeded to improve another result of Hirsch. Denote the sum of the absolute values of the elements of the ith row by S_i and the corresponding sum for the jth column by T_j, set $\max S_i = S$ and $\max T_j = T$. For each root λ of the matrix we have $|\lambda| \leqq \frac{1}{2}(S + T)$. This result was later improved by W. V. Parker,[7] one of Browne's former students, now Head of the Department of Mathematics at the Alabama Institute of Technology. When Browne was in Europe in 1946, teaching at Universities of the Army, I had to substitute for him here in giving the course on matrices. I had never given this course before nor had I published any paper in this field. Since I wanted to consider some newer papers in my class, it was natural that I studied some of Browne's papers, in particular this paper and related results; I became highly interested in this paper and using a method I knew from a paper of Rohrbach[8] I could improve

[2] W. M. Whyburn, "An Existence Theorem for Differential Systems."—W. M. Whyburn, "On the Functional Relation Between the Solutions of Differential Systems and the Initial Conditions."—J. W. Lasley, Jr., "Note on the Line Equation of a Plane Curve."—E. L. Mackie, "The Jacobi Condition for a Problem of Mayer with Variable End Points."

[3] University of Chicago Press, 1929, pp. 44–47.

[4] I. Bendixson, "Sur les racines d'une équation fondamentale," Acta Mathematica, v. 25 (1902), pp. 359–366.

[5] A. Hirsch, "Sur les racines d'une équation fondamentale," Acta Mathematica, v. 25 (1902), pp. 367–370.

[6] T. J. I'A. Bromwich, "On the roots of the characteristic equation of a linear substitution," Acta Mathematica, v. 30 (1906), pp. 297–304.

[7] W. V. Parker, "The characteristic roots of a matrix," Duke Mathematical Journal, v. 3 (1937), pp. 484–487.

[8] H. Rohrbach, "Bemerkungen zu einem De-

some of Browne's results. This was the starting point of my own work in the theory of matrices. For this inspiration I have to be very grateful to Browne. Of course, I had expressed my gratitude to him earlier, but when I visited him the last time, in the hospital three days before he died, the conversation was mostly between Mrs. Browne and myself while he was attentively listening. We discussed the just published excellent review of his book by the English algebraist, W. L. Ferrar,[9] and Mrs. Browne raised the question of how I had become interested in the same fields. So I could tell Mrs. Browne in his presence what an important role her husband's work had played in my own life.

An excellent history of the results on bounds for the characteristic roots were given by Browne in an invited address before the Mathematical Association at Williamsburg in 1938 [20].

Another of Browne's papers I used in my own work is the paper, "On the separation property of the roots of the secular equation" [8]. Let $f(x)$ be a polynomial with only real zeros. It is well known that between two roots of $f(x)$ there always lies a zero of $f'(x)$. A zero of multiplicity k of $f(x)$ is a zero of multiplicity $k - 1$ of $f'(x)$. Weber,[10] in his book on Algebra proved a similar theorem in 1898. Let $L_i(x)$ be the characteristic function of an Hermitian matrix of order i and $L_{i-1}(x)$ the characteristic equation of a principal minor, then the roots of $L_{i-1}(x) = 0$ separate the roots of $L_i(x) = 0$. It is clear what is meant if all the roots of $L_i(x) = 0$ are different. But Weber did not consider the case that $L_i(x)$ has multiple zeros. This case is studied carefully by Browne. It can happen that a multiple zero of order k of $L_i(x)$ is a zero of multiplicity $k - 1$, k, or $k + 1$ of $L_{i-1}(x)$. The full result is a very important extension of Weber's theorem. It cannot be easily stated. Due to the title of Browne's paper it is now called the Separation Theorem for the roots of Hermitian matrices.

My time does not allow to discuss the other papers of Browne although all of them are very interesting.

The last years of his life were filled by his work for his book, *Introduction to the Theory of Determinants and Matrices*, first published as lecture notes for his students [3]. For many years Browne had given the two courses on matrix theory. I have spoken to hundreds of students who have taken these classes and all agreed that they were excellent. Therefore, the book was already well known when it was published last May by the U.N.C. Press, and it is not surprising that already more than 1000 copies have been sold.

The review in Nature[11] which I mentioned before begins, "This is a good book, well written and well printed. It covers the theory of matrices, including their application to algebraic forms, and it covers that theory carefully and thoroughly. A novice reading it may find the going heavy, but the development though sometimes severe is logical, clear and fully stated." The review of W. Givens in the American Mathematical Monthly[12] closes as follows: "This book has enough novelty in content and treatment to deserve a place in every general mathematics library. Teachers of courses in applied matrix algebra may well consider it seriously as a possible text and will certainly want a copy as a source of examples of alternative and often excellent proofs of standard results and of supplementary material usually not included in a first course."

Browne directed five Ph.D. dissertations and 25 master's theses. Some of his former students are now first class mathematicians, teaching at U.N.C. and other institutions. In addition, most of our graduate students have taken at least one of Browne's courses and have profited greatly by them.

Browne was an excellent mathematician. There is no doubt that he would have published many more papers if this lingering illness had not stricken him so early. Moreover, it was his conviction that we have two duties, research and teaching, teaching not only of graduate students, but starting on the freshman level. Some of our freshmen may be the future presidents, governors, generals, and leading men in many professions. Many of them will be teachers in our school system. They all urgently need the ability of logical thinking which cannot be better taught than in a good mathematics class. Anyone who has war experience knows how important this ability is in the first battle line.

I believe we cannot better honor the memory

terminantensatz von Minkowski," Jahresbericht der Deutschen Mathmatiker Vereinigung, v. 40 (1931), pp. 49–53.

[9] Nature, v. 182 (1958), pp. 1689–90.

[10] H. Weber, Lehrbuch der Algebra, v. I (1898), pp. 295–304.

[11] L. C. 9.

[12] V. 66 (1958), pp. 247–258.

of E. T. Browne than by working in his spirit for this university with all our ability, with all our energy to make the Department of Mathematics of this university one of the best in the whole country in research and in teaching.

PUBLICATIONS OF E. T. BROWNE

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3. Introduction to the Theory of Determinants and Matrices, (Chapel Hill, University of North Carolina Press), 1958. (Published earlier in mimeographed form as lecture notes.)

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14. "On the Metric Equation $F(X) = A$ and $P(A, X) = 0$," Bulletin of the American Mathematical Society, v. **41** (1935), pp. 737–743. Abstract ibid., p. 486.
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16. "The Classification of Correlations in the Plane" (with C. A. Denson), American Mathematical Monthly, v. **44** (1937), pp. 566–573.
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INTERSECTION OF STRAIGHT LINE WITH SPIRAL

By T. F. Hickerson

Chapel Hill, North Carolina

Let x and y be the coordinates of any point on the center-line spiral (Fig. 1) referred to Point A, the TS, as origin.

R = variable radius of the center-line spiral (the base spiral) referred to any point distant l from point A (R and θ not shown).

θ = angle subtended by spiral arc AP.

At point C, the SC, arc $AC = l_s$ and θ becomes θ_s, the so-called "spiral angle" and l_s is the total length of spiral connecting a tangent with a circular curve of degree D_c.

The following are well known expressions: $\theta = (l/l_s)^2 \times \theta_s$; $l_s = 200(\theta_s/D_c)$.

Let W = radial distance from the base spiral to the outer (or inner) parallel spiral.

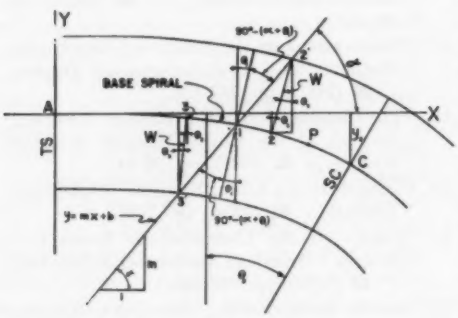


FIGURE 1

For an assigned θ, the actual values of the coordinates of a point on the base spiral are lx and ly; where x and y (for the unit spiral) are given in a Table compiled by the author, or by the expanded series:

$$x = \left(1 - \frac{\theta^2}{10} + \frac{\theta^4}{216} - \frac{\theta^6}{9360}\right); \qquad y = \left(\frac{\theta}{3} - \frac{\theta^3}{42} + \frac{\theta^5}{1320}\right). \tag{a}$$

Let the equation of the given line be designated by $y = mx + b$, or

$$y - y'' = m(x - x''), \tag{b}$$

where the origin of coordinates is at point A (the TS). Assume the line to cross the X-axis at a given point distant a from the origin, that is, at the point where ($x'' = a$, $y'' = 0$). Then Eq. (b) becomes

$$y = m(x - a). \tag{c}$$

At point 1, the intersection of the line with the base spiral, let the corresponding values of l and θ equal l_1 and θ_1, while the coordinates are $l_1 x_1$ and $l_1 y_1$ respectively.

Since both the TS and SC of the outer and inner spirals must be radially opposite the base spiral at these points (when normally they are not), then their true spiral characteristics are slightly modified thus causing a slight (but inappreciable) compounding at the SC.

From points 2′ and 3′ where the line crosses the outer and inner spirals, let radial lines of length W extend to points 2 and 3 of the base spiral. Hence (see Fig. 1), $y_2' = W \cos\theta_2 - l_2 y_2$; $x_2' = l_2 x_2 + W \sin\theta_2$; where l_2, θ_2, y_2, and x_2 apply to point 2 on the base spiral. Likewise, for the inner spiral, we have

$$y_3' = -[W \cos\theta_3 + l_3 y_3]; \; x_3' = l_3 x_3 - W \sin\theta_3 .$$

Given the following data: l_s, θ_s, W, a, m (slope of line), and α (where $\tan\alpha = m$); the coordinates of the intersection points 1, 2′, and 3′ may be derived from the following Eqs. (1), (2), and (3) [leading to Eqs. (8–10)], which are adapted to electronic computation.

Since the given line passes through points 1, 2′, and 3′, its equation is satisfied by the coordinates of these points; that is,

$$\begin{aligned} -l_1 y_1 - m l_1 x_1 + ma &= 0; \\ y_2' - m x_2' + ma &= 0, \end{aligned} \tag{d}$$

or

$$\begin{aligned} W \cos\theta_2 - l_2 y_2 \\ - m(l_2 x_2 + W \sin\theta_2) + ma &= 0; \\ y_3' - m x_3' + ma &= 0, \end{aligned} \tag{e}$$

or

$$-[W\cos\theta_3 + l_3 y_3] - m(l_3 x_3 - W\sin\theta_3) + ma = 0. \quad \text{(f)}$$

Omitting subscripts for the moment, and reducing, Eqs. (d), (e), and (f) become:

$$l(y + mx) - ma = 0; \quad (1)$$

$$l(y + mx) - [W\cos\theta - m\sin\theta) + ma] = 0; \quad (2)$$

$$l(y + mx) - [W(m\sin\theta - \cos\theta) + ma] = 0. \quad (3)$$

Substituting the expanded series for y and x in Eq. (2), we have

$$l\left[\left(\frac{\theta}{3} - \frac{\theta^3}{42} + \frac{\theta^5}{1320}\right) + m\left(1 - \frac{\theta^2}{10} + \frac{\theta^4}{216} - \frac{\theta^6}{9360}\right)\right] - [W(\cos\theta - m\sin\theta) + ma] = 0. \quad (4)$$

For an approximation, Taylor's Theorem (given in treatises on the calculus) will be used. Applying this theorem to Eq. (4), let $\theta_2 = \theta + K$, where θ is the solution, i.e., $f(\theta) = 0$ and θ_2 is an approximation. Then

$$K \times f'(\theta) = f(\theta_2) = E, \quad (5)$$

where E is the error.

Noting that $l = (l_s/\sqrt{\theta_s})(\sqrt{\theta})$, differentiating Eq. (4), neglecting terms θ^5 and over, and reducing, we get

$$f'(\theta) = (l_s/\sqrt{\theta_s})[\tfrac{1}{2}\theta^{1/2} - \tfrac{1}{12}\theta^{5/2} + m(\tfrac{1}{2}\theta^{-1/2} - \tfrac{1}{4}\theta^{3/2} + \tfrac{1}{48}\theta^{7/2})] + [W(\sin\theta + m\cos\theta)] = E. \quad (6)$$

For this approximation put $\theta = \theta_2$ and divide both sides of Eq. (5) by $\sqrt{\theta}$ or $\sqrt{\theta_2}$. Then

$$K[(l_s/\sqrt{\theta_s})[\tfrac{1}{2} - \tfrac{1}{12}\theta_2^2 + \frac{m}{\theta_2}(\tfrac{1}{2} - \tfrac{1}{4}\theta_2^2 + \tfrac{1}{48}\theta_2^4)] + (W/\sqrt{\theta_2})[\sin\theta_2 + m\cos\theta_2]] = (l_s/\sqrt{\theta_s})[\tfrac{1}{3}\theta_2 - \tfrac{1}{42}\theta_2^3 + \tfrac{1}{1320}\theta_2^5 + m(1 - \tfrac{1}{10}\theta_2^2 + \tfrac{1}{216}\theta_2^4 - \tfrac{1}{9360}\theta_2^6)] - (1/\sqrt{\theta_2})[W(\cos\theta_2 - m\sin\theta_2) + ma]. \quad (7)$$

Eq. (7) can be solved directly and the correction K found. The right-hand side of Eq. (7) has to be taken to more terms since greater accuracy is required, for this must eventually be computed to almost zero, whereas the coefficient of K in the left-hand side remains comparatively large, and as K approaches zero, such

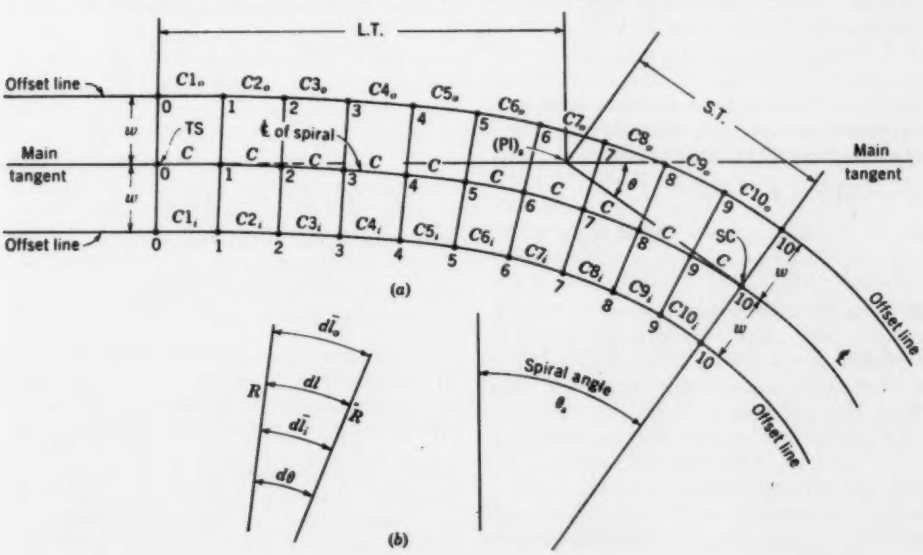


FIGURE 2

accuracy is not required. Then $\theta = (\theta_2 - K) =$ recurring value of θ_2. For the sake of brevity, multiply by $\sqrt{\theta_s}$ and let

$$C_1 = l_s\left[\frac{1}{2} - \frac{1}{12}\theta_1^2 + \frac{m}{\theta_1}\left(\frac{1}{2} - \frac{1}{4}\theta_1^2 + \frac{1}{48}\theta_1^4\right)\right];$$

$$C_2 = l_s\left[\frac{1}{2} - \frac{1}{12}\theta_2^2 + \frac{m}{\theta_2}\left(\frac{1}{2} - \frac{1}{4}\theta_2^2 + \frac{1}{48}\theta_2^4\right)\right] + W(\sqrt{\theta_s}/\sqrt{\theta_2})[\sin\theta_2 + m\cos\theta_2];$$

$$C_3 = l_s\left[\frac{1}{2} - \frac{1}{12}\theta_3^2 + \frac{m}{\theta_3}\left(\frac{1}{2} - \frac{1}{4}\theta_3^2 + \frac{1}{48}\theta_3^4\right)\right] + W(\sqrt{\theta_s}/\sqrt{\theta_3})[\sin\theta_3 + m\cos\theta_3].$$

As values θ_1, θ_2, θ_3 in turn are assigned for θ, the corresponding y's and x's are y_1, x_1; y_2, x_2; y_3, x_3.

For a further simplification, let Eq. (a) represent the y's and x's in terms of special values of θ as indicated by the subscripts.

Solutions by iteration of the following Eqs. (8, 9, 10) will give the desired points of intersection 1, 2′, and 3′.

$$K\,C_1 = l_s(y_1 + mx_1) - (\sqrt{\theta_s}/\sqrt{\theta_1}) \times ma, \quad (8)$$

in which $\theta = (\theta_1 - K) =$ recurring value of θ_1.

$$K\,C_2 = l_s(y_2 + mx_2) - (\sqrt{\theta_s}/\sqrt{\theta_2})[W(\cos\theta_2 - m\sin\theta_2) + ma], \quad (9)$$

in which $\theta = (\theta_2 - K) =$ recurring value of θ_2.

$$K\,C_3 = l_s(y_3 + mx_3) - (\sqrt{\theta_s}/\sqrt{\theta_3})[W(m\sin\theta_3 - \cos\theta_3) + ma], \quad (10)$$

in which $\theta = (\theta_3 - K) =$ recurring value of θ_3.

Preliminary approximations for the θ's in applying Eqs. (8–10) are as follows:

$\theta_1 = (l_1/l_s)^2 \times \theta_s$, where $l_1 = a - (a/l_s)^3 \times y_s/m$, and $y_s = l_s(\frac{1}{3}\theta_s - \frac{1}{42}\theta_s^3 + \frac{1}{1320}\theta_s^5)$;

$\theta_2 = (l_2/l_s)^2 \times \theta_s$, where $l_2 = l_1 + W\cot(\alpha + \theta_1)$;

$\theta_3 = (l_3/l_s)^2 \times \theta_s$, where $l_3 = l_1 - W\cot(\alpha + \theta_1)$.

As each point of a bridge pier or whatnot is determined, its adjacent distances to the previously computed points may be compared with the results obtained by the use of Eq. (11), where l_0 and l_i are the arc lengths of the outer and inner curves (Fig. 2) from the *TS* to any point radially opposite known points 2 (or 3) on the base spiral.

$$l_0 = l_2 + (l_2/l_s)^2 \times W\theta_s; \qquad l_i = l_3 - (l_3/l_s)^2 \times W\theta_s; \quad (11)$$

in which θ_s is in radians [multiply by 0.017453, if in degrees].

Example. Given $l_s = 300$ ft, $\theta_s = 9°$, $W = 15$ ft, $m = 2$ ($\alpha = 63°26.0969'$), $a = 160$ ft. Required: (1) x_1, y_1; (2) x_2', y_2'; (3) x_3', y_3'.

Because of limited space, the solution will not be carried beyond the 2nd approximation for the θ's. A summary of the results follows.

(1) $l_1 = 158.81$; $\theta_1 = 2°31.3'$; $C_1 = 6959.77$; $K = -0.000030418$ (radian).

$l_1 = 158.86$; $\theta_1 = 2°31.44'$; $x_1 = 158.8291$; $y_1 = -2.3324$.

(2) $l_2 = 165.55$; $\theta_2 = 2°44.4'$; $C_2 = 6473.76$; $K = +0.00003198$ (rad.).

$l_2 = 165.49$; $\theta_2 = 2°44.3'$; $x_2' = 166.1688$; $y_2' = 12.3469$.

(3) $l_3 = 152.12$; $\theta_3 = 2°18.8'$; $C_3 = 7632.36$; $K = -0.0000004$ (rad.).

$l_3 = 152.12$; $\theta_3 = 2°18.84'$; $x_3' = 151.4896$; $y_3' = -17.0355$.

CHECK: $m = \dfrac{y_2' - y_3'}{x_2' - x_3'} = \dfrac{29.3824}{14.6792} = 2.0016$ [exact value = 2].

INVESTIGATIONS ON THE LIFE CYCLE OF *SPORIDIOBOLUS JOHNSONII*[1]

I. Irradiation and Cytological Studies

By Robert J. Laffin[2] and Victor M. Cutter, Jr.[3]

Introduction

To accommodate those yeasts which produce and violently discharge conidia Derx (1930) created the family Sporobolomycetaceae. This family contained two genera, *Sporobolomyces* and *Bullera*, the former consisting of species which produced reniform spores and red colonies and the latter, ovoid spores and cream-colored colonies. By 1948 Derx had added two more fungi to this family. *Tilletiopsis* produced thin, sickle-shaped spores while *Itersonilia* species produced reniform spores on clamp-bearing mycelia. The last addition to the family was *Sporidiobolus* described by Nyland (1948). In its yeast phase this organism produced colonies indistinguishable from those of *Sporobolomyces* but formed in addition clamp-bearing mycelia.

The family Sporobolomycetaceae is, therefore, a morphologically heterogeneous group whose members have but one characteristic in common, the ability to produce and violently discharge their conidia (ballistospores). Since the mechanism of discharge resembles so closely the drop-excretion mechanism found in the Hymenomycetes, it has been argued that this family belongs to the class Basidiomycetes. This argument is strengthened by the observation that members of the genera *Itersonilia* and *Sporidiobolus* are able to produce mycelia with typical basidiomycetous clamp connections.

However, for these fungi to be properly placed in the class Basidiomycetes it is necessary to demonstrate not only their morphologic similarities to known members of that class but also, and more importantly, the occurrence of a perfect stage. Thus far cytological investigations have revealed the occurrence of karyogamy and subsequent reduction division in only two of the five fungi. Cutter has described these phenomena in *Sporobolomyces* (1948) and *Tilletiopsis* (1949). Present knowledge indicates that these two phenomena are lacking in *Itersonilia* (Olive 1952) and there is some suggestion (Nyland 1949) that they might occur in *Sporidiobolus*. The present investigation was undertaken to demonstrate the perfect stage of *Sporidiobolus* and to establish its basidiomycetous nature.

Materials and Methods

The culture of *Sporidiobolus Johnsonii* used throughout these investigations was obtained from Dr. George Nyland and was the type culture which he used in his original morphological and cytological studies.

During these studies the medium most commonly used was Cutter's MDA agar, the composition of which is as follows: NH_4NO_3, 2 gm.; KH_2PO_4, 0.5 gm.; $MgSO_4$, 0.25 gm.; dextrose, 40 gm.; agar, 15 gm. and water, 1000 ml. The final pH after autoclaving was 4.7. When this medium was prepared using C.P. chemicals, washed agar and double distilled water, *Sporidiobolus* made excellent growth, indicating that it requires no exogenous growth factors. In addition the organism was grown on Difco Yeast Beef Agar and on Oat Digest Agar, obtained in tablet form from the Ben Venue Laboratories of Bedford, Ohio.

Single cell isolations were made by allowing ballistospores to fall on sterile MDA agar. Blocks of agar approximately 2 mm. square were cut and, when examination under the low power of the microscope showed only a single cell present, the block was transferred to a slant of MDA agar. Within 24 hours a small colony could be seen with the aid of a dissecting microscope. Those few cultures showing two, or sometimes three, colonies were discarded.

The source of ultraviolet light for irradiation experiments was a Gates "Raymaster" obtained from the Arthur H. Thomas Company of Philadelphia. This lamp radiates principally at a wave

[1] Condensed from a dissertation submitted by the senior author to the faculty of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Microbiology.

[2] Department of Microbiology, The Creighton University School of Medicine, Omaha, Nebraska.

[3] Department of Biology, The Woman's College of the University of North Carolina, Greensboro, North Carolina.

length of 2537 Å. Cells to be irradiated were washed from the surface of a 24 hour old MDA slant with distilled water. The cell concentration was determined by means of a hemacytometer and adjusted for irradiation to approximately 10^6 cells per ml. The suspension was agitated for several minutes to break up any clumps of cells and then irradiated with constant agitation in a thin layer in a Petri plate situated 40 cm. from the lamp. Samples were removed at regular intervals and diluted before being plated on yeast beef agar. The plates were incubated at room temperature for 48 hours, after which colony counts were made. To verify the results obtained by this technique, a known haploid strain of *Saccharomyces cerevisiae* was likewise irradiated.

In conducting the cytological investigations the method of choice was a modification of the acid-Giemsa technique. The procedure is described below.

1. Fasten cells to slide with Mayer's albumin-glycerine adhesive
2. Fix in vapor of 2% osmic acid, 4 minutes
3. Fix in Schaudin's fluid (saturated aqueous $HgCl_2$ 50 parts, absolute alcohol 25 parts, glacial acetic acid 2 parts) for 5 minutes
4. Rinse through 70% alcohol, 50%, 30%, 10% to distilled water
5. Hydrolyze in 1 N HCl at 60° C. for 7 minutes
6. Rinse in distilled water
7. Rinse in phosphate buffer pH 7 for 1 minute
8. Stain in Giemsa solution 50 to 60 minutes (Giemsa solution was 4 ml. of stock solution made up as directed by manufacturer in 60 ml. phosphate buffer pH 7)
9. Rinse in distilled water
10. Dehydrate rapidly through acetone-xylene mixtures as follows:
    acetone 75-xylene 25
    acetone 50-xylene 50
    acetone 25-xylene 75
    Then two changes of pure xylene
11. Mount in neutral Canada balsam in xylene

The manner of fastening the cells to the slide varies, depending on whether one is staining yeast cells or mycelium. It was found that yeast cells would stick quite readily to slides coated with a thin layer of adhesive. Such preparations had to be placed in the osmic acid vapor immediately because drying, even for very short periods, caused a distortion of the cytoplasm and its contents. In the case of the mycelium, material for staining was obtained by growing the fungus on cellophane placed on MDA agar. After four to five days the small mycelial mats could be readily removed from the cellophane. Macerating for one minute in a Waring blendor yielded a suspension of mycelial fragments suitable for staining. Several drops of suspension were pipetted onto a slide and allowed to dry. Unlike the yeast cells, the mycelial cells were not adversely affected by drying.

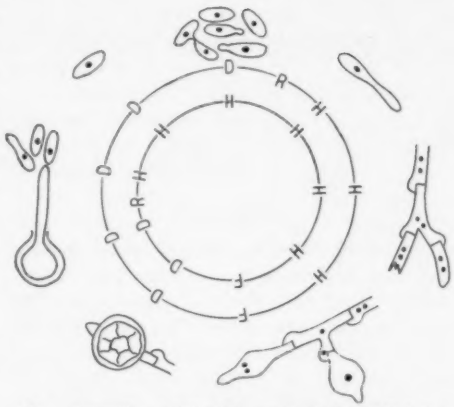


FIG. 1. Outline of the life cycle of *Sporidiobolus Johnsonii* and proposed nuclear histories.

OBSERVATIONS AND DISCUSSION

An outline of the life cycle of *Sporidiobolus* as reported by Nyland is presented in Fig. 1. Starting from a vegetative yeast cell, this fungus buds in a yeast-like manner and also produces ballistospores aerially on sterigmata. These ballistospores may in turn bud or produce other aerial spores on sterigmata. All of these cells are uninucleate. When a culture is approximately three days old, mycelium can be seen growing out from the edge of the colony. The mycelium is septate with a clamp connection at almost every septum and cytological investigation reveals that the hyphal cells are binucleate. As the culture ages still more, resting spores are formed on short lateral branches. The stalk bearing the spore is equipped with a clamp connection and the spore itself has a hyaline, hyphal projection extending from its apex. Young spores are hyaline and thin-walled but when they reach maturity, a thick endospore and thin golden-brown exospore are present. Upon germination, the resting cells produce a short germ tube from which yeast-like cells are budded.

Nyland believed that the yeast cells and ballistospores were diploid, that the mycelium was

dikaryotic and haploid and that karyogamy occurred in the immature resting cell. To illustrate such a nuclear history use is made in Fig. 1 of the letters "D" and "H" to denote the diploid and haploid phases respectively. The letter "F" signifies the point at which nuclear fusion occurs. Although Nyland did not hazard any guess as to the site of reduction division, the only place where it could logically occur in such a scheme is prior to the appearance of the mycelium. This is indicated by the letter "R".

The forms assumed by *Sporidiobolus* also bear striking resemblance to those seen among the Ustilaginales. Members of this group are characterized by the production of smut-spores or chlamydospores which constitute the zygote and are typically the only diploid cells in the life cycle. During chlamydospore germination, meiosis occurs in a short hypha, the promycelium, from which haploid cells, the sporidia, are budded off. The sporidia multiply saprophytically as discrete, yeast-like cells. Under suitable conditions haploids of compatible mating-types fuse, giving rise to dikaryotic mycelium which reproduces by conjugate nuclear division. The life cycle is completed by production of chlamydospores within which the two nuclei fuse to form a diploid nucleus. This nuclear cycle as applied to *Sporidiobolus* is illustrated in the inner circle of Fig. 1. Here, too, the immature resting cell is the site of karyogamy but now meiosis occurs upon germination of the spore instead of later in the life cycle.

It was reasoned that single cell isolations would provide evidence of the ploidy of the yeast cells. Failure to obtain dikaryotic mycelium in cultures started from a single cell would suggest that the yeast cells were haploid. However, the appearance of mycelium could be explained in one of two ways. If the yeast cells were diploid, the occurrence of meiosis would produce the haploid nuclei later found in the hyphal cells. On the other hand cases are known among the Basidiomycetes in which anastomosis may occur between hyphae originating from a single basidiospore, the two nuclei in the resulting mycelium being genetically identical.

When ten cultures were started from single cells, it was observed that in each of the cultures typical clamp-bearing mycelium appeared after several days' incubation. Essentially the ploidy of the yeast cells was still unknown. It was felt that irradiation studies would provide the necessary information.

When certain micro-organisms are exposed to sources of irradiation, they show logarithmic survival curves in which a constant per cent of the cells are killed with each additional increment of dose. Such observations were interpreted by early investigators in terms of a single essential molecule or sensitive volume, an ionization or excitation which would kill the cell. On the basis of a vast number of determinations which showed that bacterial death was generally exponential, Rahn (1929) concluded that the death of a bacterial cell was due to a single event, probably the destruction of a gene leading to recessive lethal mutation.

More difficult to explain were the survival curves obtained when such materials as tissue cells, yeasts and protozoa were exposed to radiation. In such cases the curves were not exponential but sigmoid in shape. The most convincing explanation of the sigmoid survival curve is that it represents genetic replication. To exclude uncontrolled variables, such as differences in cytoplasm, Beam (1950) conducted investigations of the effect of radiations on organisms which belonged to the same taxonomic species but differed in the number of nuclei per cell. His materials were *Neurospora crassa* microconidia and macronconidia, in which the number of nuclei was 1 and 4–5 respectively and haploid and diploid strains of *Saccharomyces cerevisiae*. His figures show that *Neurospora* microconidia and haploid strains of *Saccharomyces* are killed exponentially while the survival curves for the macroconidia and diploid yeast strains are sigmoid in shape. Beam concludes that "the radiation death curve constitutes a valid test for genome number in microorganisms in which ploidy or nuclear number cannot be directly determined."

Experiments were conducted to obtain survival curves following exposure of yeast cells of *Sporidiobolus* and of a haploid strain of *Saccharomyces* to ultraviolet light. The results of such experiments are presented in Fig. 2 where the data are plotted semilogarithmically as per cent survivors against time. The curve for *Sporidiobolus* shows the broad plateau characteristic of curves for diploid and polyploid organisms. The survival curve for the haploid strain of *Saccharomyces* is exponential. It was concluded from these studies that the methods employed yielded results quite comparable to those obtained by other workers and that the yeast cells of *Sporidiobolus* contain more than the haploid set of chromosomes.

The observations of Cutter indicate that in

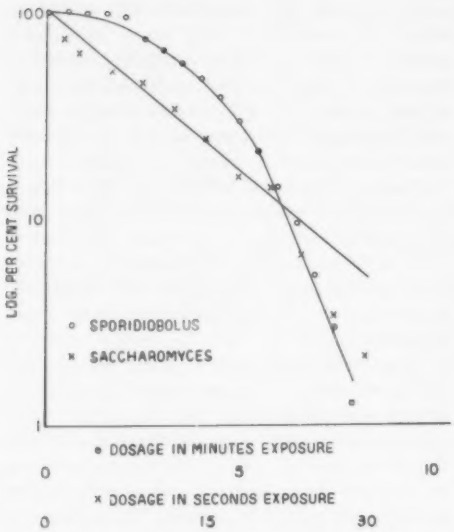


FIG. 2. Ultra-violet survival curves for *Sporidiobolus Johnsonii* and a haploid strain of *Saccharomyces cerevisiae*.

Sporobolomyces some yeast cells are capable of acting like much reduced basidia, i.e., a single nucleus (presumably diploid) undergoes two consecutive divisions, as a result of which four daughter nuclei are produced. These nuclei migrate singly into ballistospores which are produced on sterigmata, just as the haploid nuclei migrate from the basidium into the basidiospores in typical basidiomycetes. Because of the fact that the yeast phase of *Sporidiobolus* and that of *Sporobolomyces* are indistinguishable, it was reasoned that certain yeast cells in the former might also be the site of reduction division.

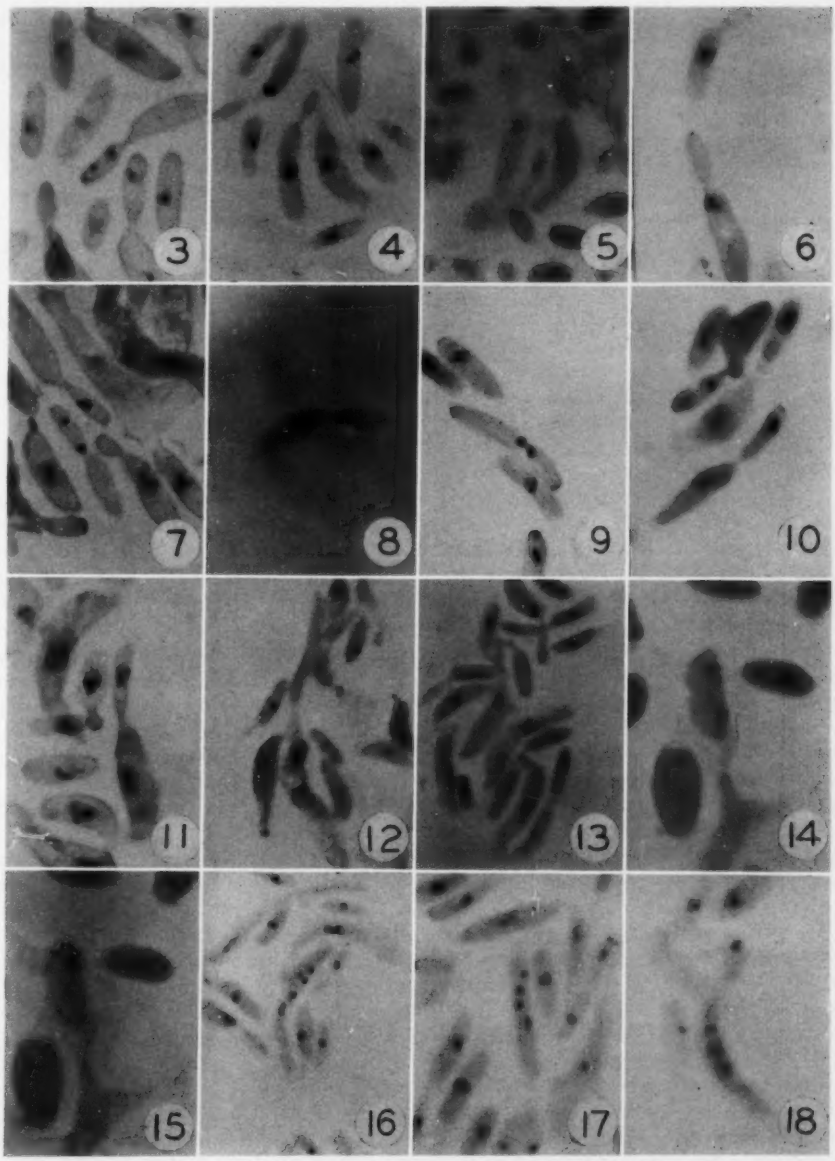
Therefore experiments were undertaken to isolate single ballistospores that might contain the products of reduction division, i.e., haploid nuclei. It was expected that if *Sporidiobolus* was heterothallic, cultures started from haploid ballistospores would fail to show the presence of clamp-bearing mycelium. Although numerous single ballistospores were isolated and the isolation technique was carried out at regular intervals for a period of 48 hours, none of the cultures failed to develop typical basidiomycetous mycelium.

In his reports on the life cycle of *Sporidiobolus* Nyland described the production of ballistospores on sterigmata on the mycelium. Since a number of cases have been reported among the Basidiomycetes in which such cells arise by a process of dediploidization, it was felt that the haplophase of *Sporidiobolus* might be readily isolated by a study of these mycelial ballistospores. Investigation revealed, however, that ballistospores could never be obtained from young mycelium. Rather, such ballistospores appeared only when mature resting cells were present on the mycelium, suggesting that the germination of these resting spores was giving rise to the yeast-like growth.

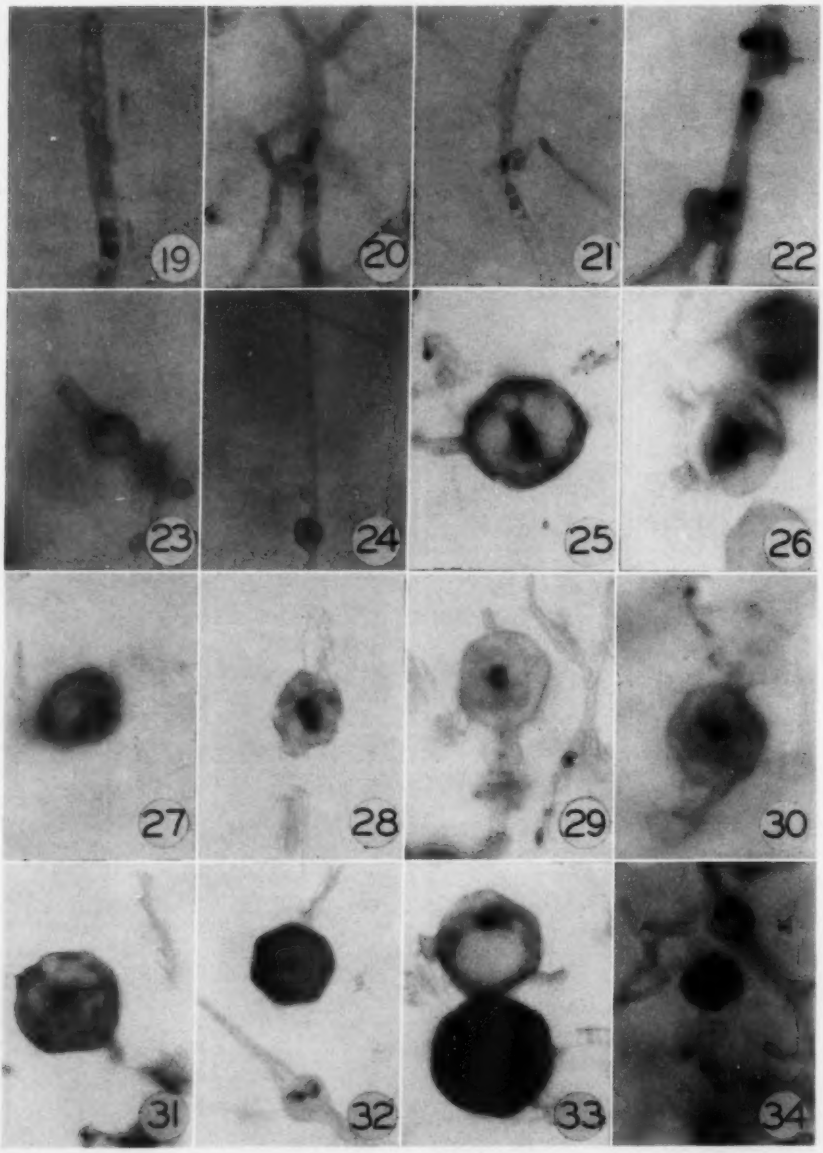
At this point cytological studies were made not only to confirm the findings of Nyland but also to demonstrate, if possible, the occurrence of reduction division. The results of these studies are presented in Figs. 3 through 34.

Yeast cells of *Sporidiobolus* are predominantly uninucleate and in budding and producing ballistospores they show typical fungal mitotic figures. The process of budding and the associated nuclear events are to be seen in Figs. 3 through 11. Interphase nuclei are spherical to oval in shape and are not as intensely stained as nuclei undergoing division (Fig. 3). As the bud starts to form, the nucleus becomes more intensely stained and irregular in appearance. In some cells it is possible to distinguish chromatin threads in these prophase nuclei (Fig. 4). The chromatin threads continue to thicken and shorten and at metaphase one can sometimes distinguish thickened, densely-stained rod-shaped structures lying side by side (Fig. 5). At other times the metaphase chromosomes appear merely as darkly-stained masses (Fig. 6). These structures may take up a position in the parent cell near the constriction between it and its bud or they may be found at times in the bud itself (Fig. 7). When the chromosomes begin to pull apart during early anaphase, dumbbell-like figures can be observed (Figs. 8 and 9). As anaphase progresses these chromosomes move even farther apart (Fig. 10). Finally one can observe during early telophase the occurrence of two darkly-stained masses, one in the parent cell and one in the bud. As telophase progresses, the chromosomes elongate and decrease in thickness, so that when the bud is almost fully formed, both its nucleus and that of the parent cell are lightly and uniformly stained and oval in shape (Fig. 11).

Nyland had reported observing occasional binucleate yeast cells during his cytological studies. Such cells were also observed during the present studies and were particularly suspect because they usually differed in morphology from most



FIGS. 3–18. *Sporidiobolus Johnsonii*. Fig. 3. Interphase nucleus. Fig. 4. Prophase nucleus. Figs. 5–7. Metaphase nuclei. Figs. 8–9. Dumbbell-like figures observed during early anaphase. Fig. 10. Late anaphase. Fig. 11. Telophase nuclei. Fig. 12. Prophase II. Fig. 13. Metaphase II. Figs. 14–15. Two focal planes of the same cell showing early anaphase II. Figs. 16–18. Telophase II, showing four linearly arranged nuclei. (Original magnification ×2500, reduced to ca. ×1875)



FIGS. 19–34. *Sporidiobolus Johnsonii*. Fig. 19. Hyphal nuclei in prophase. Figs. 20–21. Conjugate nuclear division. Fig. 22. Binucleate hyphal cell. Fig. 23. Binucleate resting cell in the process of formation. Fig. 24. Later stage of resting cell formation. Figs. 25–26. Binucleate resting cell showing formation of spore wall. Figs. 27–28. Spores in which nuclei are about to fuse. Figs. 29–30. Nuclear fusion. Fig. 31. Spore containing fusion nucleus. Fig. 32. Spore in which the nucleus is somewhat obscured by staining of the spore wall. Fig. 33. Comparison of mature and immature resting cells. Fig. 34. Typical clamp connection with septa at right angles. (Original magnification ×2500, reduced to ca. ×1875 except figure 24 which was ×1300, reduced to ca. ×975)

of the uninucleate yeast cells. In his investigations on *Sporobolomyces*, Sainclivier (1951a, 1951b, 1952) reported observing on several occasions the occurrence of conjugate nuclear division. During this process, when the cell is sufficiently wide, two haploid nuclei undergo simultaneous mitoses, the long axes of the spindles being parallel to one another and in the long axis of the cell. Such division figures were never observed in *Sporidiobolus* yeast cells. The conclusion was drawn that the two nuclei found in various cells did not represent two conjugate nuclei but rather were the products of the first division of meiosis. Support for this conclusion was provided by the later observation of cells in which two simultaneous divisions were occurring. The chromatic masses were so oriented that, after telophase, four nuclei were arranged linearly within a yeast cell, indicative of meiosis rather than conjugate nuclear division.

The presence of two nuclei within one cell is shown in Fig. 12. This binucleate cell is greatly elongated and the nuclei appear to be in prophase II. Somewhat shortened and thickened chromatin threads are discernible. Fig. 13 shows a clear metaphase II in which two groups of darkly-stained rods are visible. With the beginning of anaphase II the daughter chromatids direct themselves toward opposite poles. Figs. 14 and 15, which represent two focal planes of the same cell, show a slightly asynchronous division in which one pair of chromatids has started to pull apart while the second pair remains together. At telophase II the four reduced nuclei are arranged linearly within the cell (Figs. 16–18). This observation of meiotic-like figures with the resulting production of four nuclei supports the assumption that reduction division occurs in the yeast cell prior to the appearance of mycelium.

Since *Sporidiobolus* exists in both a yeast and a mycelial phase, a cytological investigation had necessarily to include a study of nuclear phenomena in the mycelium. It was particularly important to determine whether the formation of the clamp connection and the associated conjugate division were typical of Basidiomycetes in general and whether nuclear fusion occurred in the resting cell. The methods for preparing and staining the mycelium have already been described. The results obtained are shown in Figs. 19–34.

Fig. 19 shows a growing hyphal branch in which one can observe the presence of two nuclei. These nuclei are in prophase and darkly-stained chromatin threads are readily discernible. In addition a clamp connection is beginning to form and is distinguished as a slight bulge in the hypha opposite the distal nucleus. In Fig. 20 there are actually two simultaneous conjugate divisions occurring. The clamp associated with the distal conjugate pair has formed opposite the penultimate nucleus while the clamp associated with the proximal conjugate pair lies in a higher focal plane above the second nucleus from the bottom. The nuclei stain darker as they approach metaphase and the clamps are almost completely formed. As anaphase commences, the daughter nuclei begin to pull apart and this separation is easily observed in Fig. 21. One of the nuclei of the distal pair is seen migrating into the clamp connection. Fig. 22 shows two uniformly stained interphase nuclei lying in a hyphal cell between two clamp connections.

These studies also served to elucidate the mechanism of formation of the resting cell as well as the nuclear events associated therewith. Resting cells are formed on lateral branches of the mycelium and appear first as swellings of the hypha, containing the two nuclei found in the hyphal tip. Fig. 23 shows a resting cell in the process of formation, with the two nuclei readily discernible. In addition one can distinguish the hyphal projection extending from the apex of the spore. A later stage is shown in Fig. 24. The spore is binucleate and has a hyphal projection at its apex. The hypha bearing the spore is equipped with a typical clamp connection and two nuclei are clearly visible in the hyphal cell. As the binucleate resting cell matures, a thick wall is formed. Two such cells are seen in Figs. 25 and 26. Resting cells in which the two nuclei are about to fuse appear in Figs. 27 and 28. In Fig. 27 the two centrioles associated with the nuclei are clearly seen. Only one of the centrioles is visible in Fig. 28. As karyogamy progresses, the two nuclei become flattened on their apposing sides as they approach one another (Figs. 29 and 30). Once fusion is complete, the resting cell contains a single large nucleus (Fig. 31).

It becomes virtually impossible to demonstrate any nuclear structure when these resting cells reach maturity. The cell wall takes up a large amount of the stain and obscures the contents of the spore. Fig. 32 shows a spore in which the single nucleus is barely visible. Fig. 33 illustrates the difference between a mature resting cell and

one in which the thick wall has not yet been completely formed. Fig. 34 is included to show the typical clamp connections associated with the resting cell. The two septa which are formed at right angles to one another and across the spindles of the conjugately-dividing nuclei are readily seen.

SUMMARY

The present studies have served to confirm earlier reports on the forms assumed by *Sporidiobolus Johnsonii* during its life cycle. The ability of single yeast cells to give rise to cultures in which dikaryotic mycelium was observed suggested that the yeast cells were diploid. This assumption was tested by plotting a survival curve after exposure of yeast cells to ultra-violet light. The curve was clearly sigmoid in shape, indicating that yeast cells of *Sporidiobolus* contain more than the haploid set of chromosomes. To confirm the validity of these results, a known haploid strain of *Saccharomyces* was irradiated and the survival curve obtained was exponential.

Attempts to prove the occurrence of meiosis in *Sporidiobolus* by securing cultures of haploid yeast cells were uniformly unsuccessful. Subsequent observations indicated that this failure might be attributed to the fact that reduction division occurs only rarely.

Cytological investigations proved more rewarding and confirmed earlier reports on the nuclear condition of yeast and hyphal cells. Typical mitotic figures were observed in budding yeast cells. In addition the occurrence of meiotic-like figures was noted and yeast cells containing four linearly arranged nuclei were seen.

The process of clamp connection formation and the associated conjugate nuclear division were followed in a study of the mycelium. These phenomena in *Sporidiobolus* are identical to the same events seen in typical Basidiomycetes. A close study of resting cell formation revealed that this spore is binucleate during its entire development and becomes uninucleate only after karyogamy, which usually takes place after the thick spore wall has been formed.

On the basis of these findings it is proposed that *Sporidiobolus Johnsonii* be classified as a Basidiomycete and that its nuclear history can best be described by the scheme in the outer circle in Fig. 1. Material to be presented in a subsequent paper will lend further support to this proposal.

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INVESTIGATIONS ON THE LIFE CYCLE OF *SPORIDIOBOLUS JOHNSONII*[1]

II. Mutants and Micromanipulation

By Robert J. Laffin[2] and Victor M. Cutter, Jr.[3]

Introduction

When Derx (1930) created the family Sporobolomycetaceae, he included in it those fungi which produce and violently discharge conidia. The resemblance between the mechanism of discharge and the drop-excretion mechanism found in the Hymenomycetes suggested that the members of this family should be classified as Basidiomycetes. This argument was strengthened by Nyland's description (1948) of *Sporidiobolus Johnsonii*. During its life cycle this fungus buds in a yeast-like manner and produces ballistospores on sterigmata. In addition, aging cultures show the presence of hyphae bearing typical basidiomycetous clamp connections.

Cytological studies by Nyland showed that the yeast cells are predominantly uninucleate, that the cells in the mycelium are typically binucleate and that karyogamy occurs in resting cells formed on the mycelium. He made no attempt to determine the site of reduction division.

Investigations by Laffin and Cutter (1959) confirm Nyland's report that cultures started from single yeast cells ultimately showed the presence of dikaryotic mycelium, suggesting that the yeast cells are diploid. Irradiation studies of *Sporidiobolus* produced a sigmoid survival curve typical of organisms containing more than the haploid number of chromosomes. When cytological examination showed meiotic-like nuclear figures in some yeast cells, of conjugate nuclear division in the hyphae and of karyogamy in the resting cells, it was proposed that *Sporidiobolus Johnsonii* be classified as a Basidiomycete.

The current investigation was undertaken to provide further evidence in support of this proposal.

Materials and Methods

In attempting to isolate biochemical mutants, suspensions of yeast cells were exposed to a source of ultra-violet light for varying lengths of time. The survivors were diluted and incorporated into MDA agar pour plates. Those non-mutants which were capable of growth on this minimal medium produced visible colonies within several days. These colonies were marked by placing a spot of India ink on the bottom of the Petri plate and then the plate was layered with yeast-beef agar. Organisms capable of multiplying only upon addition of exogenous growth factors were picked to slants of yeast-beef agar for subsequent testing to determine their specific growth factor requirements.

Efforts were made to isolate monokaryotic cells by performing micrurgical operations upon dikaryotic, clamp-forming mycelium. Essentially the micro-operation consists of puncturing certain cells during the process of clamp connection formation and allowing a single monokaryotic cell to survive. This technique, first described by Harder (1927), was refined by Aschan (1952) and excellent drawings to illustrate the procedure are to be found in the latter paper.

A deFonbrune micromanipulator was used for the operations. The only microtool required was a fine glass needle, which was drawn from a piece of glass rod. To prepare cells for the operation, growth from a four to five day old culture on an MDA slant was macerated for one minute in approximately 20 ml. of water in a Waring blendor. A small amount of the resulting suspension of mycelial fragments was pipetted onto a piece of cellophane previously placed upon the surface of a plate of MDA agar. Within two days small colonies began to appear and an entire colony could be removed from the plate by cutting a small piece of cellophane 3–4 mm. square from the large sheet.

To prevent the preparation from drying out,

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the small square of cellophane was laid on a thin sheet of agar approximately 10 mm. square and less than 1 mm. thick. Such a preparation is sufficiently thin so that the hyphae are in sharp focus even though viewed through cover slip, agar and cellophane and magnified 660 times.

The advantage of having a whole colony on the small square of cellophane lies in the fact that numerous hyphal tips are available. Following the operation the penultimate cell was severed from a mycelial tip and all superfluous cells removed. As many as five or six such cells could be isolated from a single colony and their further progress followed by transferring the cellophane to a Petri plate containing oat digest agar. These monokaryotic cells will be referred to hereafter as operation cells.

When it became necessary to study single, germinating resting cells, difficulty was encountered because of the fact that the percentage of cells which actually germinate is extremely small. This difficulty was overcome by plating large numbers of cells (400–500) but under conditions which permitted the isolation of any single cells observed germinating.

A Petri plate of MDA agar was marked off with a glass marking pencil into 15 mm. squares. Suspensions of resting cells were prepared so that a single drop delivered into the center of a square contained 5–6 cells. The drop was sufficiently small so that its entire contents could be viewed with the low power of the microscope.

Those few resting cells which germinated did so within 24 hours by producing a germ tube from the end of which yeast cells budded. The yeast cells were allowed to multiply for an additional 24 hours and then washed from the agar square onto a plate of MDA agar to which thiamin had been added. These plates were in turn incubated to obtain colonies for subsequent study.

Observations and Discussion

Evidence from irradiation studies had indicated that *Sporidiobolus* yeast cells were diploid in which case attempts to isolate biochemical mutants would meet with little success. The occurrence of a recessive gene mutation in a diploid cell is normally masked by the presence within that cell of the dominant allele of this gene, whether this allele be situated within the same nuclear membrane (heterozygote) or another nuclear membrane (heterokaryon). However, the observation of a process resembling meiosis in yeast cells suggested that cells containing haploid nuclei might be isolated by the use of a suitable detection technique.

Yeast cells of *Sporidiobolus* were irradiated and incorporated into plates of minimal medium. Colonies appearing after several days were marked before layering the plates with yeast-beef agar. Although no new colonies appeared upon reincubation, it was noted on occasion that a pre-existing small colony had increased markedly in size. When yeast cells from these enlarged colonies were tested to determine their specific growth factor requirements, it was found in each case that growth was directly affected by the addition of thiamin to the medium. No other vitamin or amino acid requiring mutants were ever isolated.

While it appeared at first as if cultures of haploid yeast cells had been obtained which required thiamin for normal growth, continued incubation of these isolates resulted eventually in the appearance of mycelium which was equipped with clamp connections and upon which resting cells eventually developed. Since earlier studies had indicated that mycelium was produced only in cultures started from diploid cells, an explanation was sought for the ability of this putative diploid culture to show a biochemical loss mutation.

The suggestion that the mutation to thiamin requirement was dominant to thiamin independence was discarded as improbable. It seemed more likely that the culture of *Sporidiobolus* used in these studies was heterozygous for thiamin independence. Thus mutation of the dominant allele would give rise to a homozygous recessive culture.

It was suggested that this hypothesis could be simply tested by a "selfing" of the original strain. If it was indeed heterozygous, one would expect to obtain thiaminless segregants. When a culture of the mycelial phase alone was prepared and allowed to mature, numerous resting cells were formed and these in turn were germinated by suspending them in water for several days. The resulting yeast cells were plated on MDA agar plus thiamin and the number of colonies was counted. Utilizing the replica plating technique (Lederberg and Lederberg, 1952), samples were removed from each colony and subcultured on both MDA alone and MDA plus thiamin. Examination of these replicate plates after several days' incubation revealed consistently one-fourth fewer colonies on unsupplemented

MDA, indicating the failure of homozygous recessive segregants to grow in the absence of the vitamin. This finding of thiamin requiring segregants presented the possibility of determining conclusively the site of reduction division.

A study was made of eight single germinating resting cells from the mycelial phase of *Sporidiobolus*. The yeast cells from five were uniformly thiamin independent; those from the other three were uniformly thiamin dependent. The experiment was continued by allowing all eight yeast cultures to "self". The vitamin dependent cultures uniformly yielded vitamin dependent progeny in the next yeast cycle. Of the vitamin independent cultures, two yielded only vitamin independent progeny in the next yeast cycle; the other three showed vitamin independent and vitamin dependent segregants. Schematically the results may be shown as follows:

Stock culture (Tt)
"Selfing"

2 cultures independent: (TT) "selfing"	3 cultures independent: (Tt) "selfing"	3 cultures dependent: (tt) "selfing"
All independent	Segregants for independence and requirement	All dependent

In addition to thiaminless mutants it was possible to isolate cultures of yeast cells which differed from the parent strain in their ability to produce pigment. Colonies of *Sporidiobolus* yeast cells are typically salmon-colored but occasional white or yellow colonies occur. The infrequency with which the latter occurred argued against environmental factors being responsible for their appearance. Such mutant colonies appeared with greater frequency among survivors of ultra-violet irradiation. One such isolate, which consistently produced yellow colonies, was used in subsequent micromanipulation studies along with the thiaminless mutants previously isolated. This pigment mutant was also capable of producing mycelium equipped with typical clamp connections.

When monokaryotic cells were prepared from the mycelia of the two mutant types (thiaminless and yellow), it was observed that such cells made little growth and that after 72 hours the cells appeared to be no longer viable. Although it was not possible to obtain cultures of monokaryotic mycelium, crosses between these mutant types were finally effected by mating operation cells. Approximately 75 pemultinate cells of each of the two mutant types were isolated. Examination of these cells after 48 hours revealed that only about 15 cells of each type were still viable. Using a micromanipulator, these were paired by bringing one cell of each type into contact on a thin sheet of agar. From 15 such crosses five cultures of dikaryotic, clamp-bearing mycelium were obtained. Since monokaryotic cells do not remain viable for more than 72 hours, there was little possibility that these mycelia had originated from only one operation cell or from both growing independently and the occurrence of clamp bearing mycelia was regarded as evidence of plasmogamy. Of these five cultures two were lost through contamination before the completion of single resting cell experiments.

A study of the remaining three cultures was carried out and the results are presented below. For the sake of convenience these cultures will be referred to subsequently as hybrids #1, 2 and 3. The mycelia were allowed to grow until numerous resting cells appeared. The yeast cells which appeared upon germination of these spores were tested for their thiamin requirements and ability to produce pigment.

Resting cells of hybrid #1 germinated to produce yeast cells all of which gave rise to yellow colonies and were thiamin independent. These results were observed when large numbers of resting cells were examined and were duplicated when the investigation was extended to single germinating resting cells. All of the yeast cells from any one spore were vitamin independent and produced yellow colonies on MDA agar.

The yeast cells of hybrid #2 were thiamin dependent and produced yellow colonies; those of hybrid #3 were thiamin dependent and produced salmon-colored colonies. Here again the results obtained from experiments with large numbers of spores were duplicated by single resting cell studies. It should be noted that hybrids #1 and #3 resemble, with respect to vitamin requirement and pigmentation, the parental yeast cultures which gave rise to the mycelium from which the operation cells were obtained. Hybrid #2, however, showed the recessive characteristic of both parents.

The investigation was continued by "selfing" the yeast cells of each of the three hybrids and determining the thiamin requirement of the yeast cells in the next cycle. Cells of hybrid #1

had resulted from a cross between a thiamin-independent parent which produced yellow colonies and a thiamin requiring parent which was pink. Such cells would have to be heterozygous for vitamin requirement. "Selfing" of this culture showed the occurrence of thiaminless segregants in the next yeast cycle in exactly the same manner that such segregants appeared when the stock culture was "selfed".

On the other hand cells of hybrids #2 and #3 were thiamin dependent and therefore homozygous recessive. When these hybrids were allowed to go through their life cycle, in both cases all of the yeast cells in the next cycle were likewise thiamin dependent. These results are summarized in the following schematic diagram:

Yeast cell nuclei (2n)

P p t t — p p T t

Salmon dependent — Yellow independent

After meiosis

Heterokaryotic mycelium

Hyphal nuclei (n)

Possible operation cells: P t / p t — p T / p t

Possible progeny		Hybrids obtained
P p T t	Salmon independent	
p p T t	Yellow independent	#1
P p t t	Salmon dependent	#3
p p t t	Yellow dependent	#2

SUMMARY

Attempts to produce biochemical mutants of *Sporidiobolus Johnsonii* resulted in the isolation of yeast cells which required thiamin for growth. Cultures of these cells eventually showed the appearance of dikaryotic, clamp-bearing mycelium, suggesting that the yeast cells were diploid. It was proposed therefore that the yeast cells of *Sporidiobolus* were heterozygous for thiamin requirement. To test this hypothesis the stock culture was allowed to "self" and it was found from a study of large numbers of resting cells that thiaminless segregants appeared in the next yeast cycle.

When this investigation was extended to include an examination of single germinating resting cells, it was determined that all of the yeast cells produced by any one resting cell were identical in being either vitamin requiring or vitamin independent.

The finding of thiaminless segregants supports the contention that yeast cells of *Sporidiobolus* are diploid and heterozygous for thiamin independence. The studies of single resting cells prove that reduction division does not occur in the germinating resting cell and presumably must therefore take place in the yeast cell prior to the appearance of mycelium.

The discovery of pigment mutants of this organism led to attempts to mate such mutants with thiaminless isolates. Although cultures of monokaryotic mycelia could not be obtained, crosses were effected by mating operation cells. A study of several hybrids revealed that while one of them possessed the characteristics of one parent and a second the traits of the other parent, a third had acquired a recessive characteristic from each parent. "Selfing" of these hybrids provided further evidence that the nuclear divisions occurring during the germination of the resting cell are mitotic and not meiotic in nature.

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ADDITIONS TO THE FRESH-WATER ALGAE IN NORTH CAROLINA IV[1]

An Annotated List of Diatoms

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The fresh-water diatoms of North Carolina have received very little attention in phycological studies. Only a few investigators have attempted to identify these algae. Silva and Sharp (1944) list 20 species (all from the mountains); Phillips and Whitford (1958) list 9, and Whitford (1943) lists 8.

This paper reports a total of 184 diatom taxa, representing 38 genera. It includes the 37 mentioned by the previous authors. These are marked at the end of the particular annotation by a (1) designating Phillips and Whitford, a (2) for Silva and Sharp and a (3) for Whitford. Most of the collections by the current authors were made in brooks, creeks and rivers. However, occasional collections were made in ponds, seeps, springs and other locales. Although this list represents a small portion of the probable diatom flora, several rare or interesting species are recorded. Some of these are *Actinella punctata*, *Attheya zachariasi*, *Biddulphia laevis*, *Hydrosera triquetra*, *Tabellaria binalis* and *Terpsinoë americana*. The abbreviations CP, P and M used in the list denote the Coastal Plain, Piedmont and Mountains.

The authors wish to express their appreciation to Dr. Ruth Patrick and the Philadelphia Academy of Natural Sciences for the use of their library, herbarium and facilities during several visits and to Dr. C. W. Reimer and Dr. M. H. Hohn of that Academy for their aid in the identification of several species.

Bacillariophyceae

CENTRALES

COSCINODISCACEAE

Melosira ambigua (Grun.) O. Müll. River Plankton, Lenoir Co. CP.

Melosira granulata (Ehr.) Ralfs. Widespread in the plankton of rivers throughout the year. Common in lakes in spring. CP, P, M.

Melosira granulata var. **angustissima** Müll. With the species but frequently much more abundant. CP, P, M. (1).

Melosira granulata var. **procera** (Ehr.) Grun. Seen several times in the plankton of lakes and streams. Bladen, Martin, Stanly, Warren Cos. CP, P.

Melosira islandica O. Müll. In plankton of a lake. Burke Co. P.

Melosira italica var. **granulata** Grun. On rock in rapids of Haw River. Chatham Co. P.

Melosira italica var. **subartica** O. Müll. In a spring. Wake Co. P.

Melosira italica var. **tenuissima** (Grun.) O. Müll. In a pool and brook. Pender Co. CP.

Melosira italica var. **valida** Grun. In pond plankton. Brunswick Co. CP.

Melosira varians C. A. Agardh. Widespread, in rapids and tychoplankton of streams. CP, P, M.

Cyclotella comta (Ehr.) Kütz. Nannoplankton of Trent River and a lake. Jones and Wake Cos. CP, P.

Cyclotella meneghiniana Kütz. In several brooks and creeks. Bladen, Pender and Wake Cos. CP, P.

Cyclotella stelligera Cleve & Grun. In nannoplankton of Lake Johnson. Wake Co. P.

Stephanodiscus hantzschii var. **delicatula** Cleve. In a large pool at Raleigh. Wake Co. P.

Stephanodiscus hantzschii var. **pusilus** (Grun.) Kreiger. In the plankton of Lake James. Burke Co. P.

RHIZOSOLENIACEAE

Rhizosolenia eriensis H. L. Smith. Widespread but never abundant in the plank-

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ton of the piedmont and coastal plain. CP, P. (1, 3).

Rhizosolenia longiseta Zach. Rare in one pond. P. (1).

CHAETOCERACEAE

Attheya zachariasi J. Brun. Rare in plankton of lakes and rivers. CP, P. (1).

BIDDULPHIACEAE

Biddulphia laevis Ehrenb. Attached and in tychoplankton of the Cape Fear, Catawba, Haw, Neuse and Yadkin Rivers. CP, P.

Hydrosera triquetra Wall. Attached and in the tychoplankton of the Rocky and Yadkin Rivers, sometimes abundant. P.

ANAULACEAE

Terpsinoë americana (Bail.) Ralfs. In plankton of Pee Dee River, Anson Co. P.

PENNALES

TABELLARIACEAE

Tabellaria binalis (Ehr.) Grun. In shallows of two black water lakes. Bladen Co. CP.

Tabellaria fenestrata (Lyngb) Kütz. Abundant, cosmopolitan. Most abundant in spring. CP, P, M. (3).

Tabellaria flocculoca (Roth) Kütz. Abundant, cosmopolitan. Most abundant in spring. CP, P. M. (3).

MERIDIONACEAE

Meridion circulare Agardh. Abundant in streams throughout the state where temperatures are low. Growth is inhibited by temperatures above 15°C. CP, P, M.

DIATOMACEAE

Diatoma hiemale var. **mesodon** (Ehr.) Grun. In a seep at 6000 ft. elevation. Mitchell Co. M.

Odontidium hiemale var. **mesodon** (Ehr.) Grun. In a pool at 3000 ft. elevation. Swain Co. M. (2).

FRAGILARIACEAE

Fragilaria capucina Desmarzières. In House Creek? Wake Co. P.

Fragilaria crotonensis Kitton. In the plankton of ponds and streams. CP, P.

Fragilaria rumpens (Kütz.) Grun. In aufwuchs on dam of a pond. Wake Co. P.

Fragilaria vaucheriae (Kütz.) Boye Pet. In shallows of Trent River? Craven Co. CP.

Fragilaria virescens Ralfs. In a stream at 3200 ft. elevation. Swain Co. M. (2).

Fragilaria virescens var. **capitata** Østr. With the species. (2).

Synedra affinis Kütz. In rapids of House Creek. Wake Co. P.

Synedra affinis var. **delicatula** Grun. In plankton of pond. Wake Co. CP.

Synedra affinis var. **hybrida** Grun. In rapids of House Creek. Wake Co. P.

Synedra amphicephala Kütz. In Salmon Creek. Bertie Co. CP.

Synedra minuscula Grun. In plankton, Boone's Pond. Wake Co. P.

Synedra nana Meister. Spring seep off House Creek. Wake Co. P.

Synedra pulchella var. **lanceolata** O'Meara. On rock in rapids of House Creek. Wake Co. P.

Synedra rumpens Kütz. In Salmon Creek. Bertie Co. CP.

Synedra rumpens var. **fragilarioides** Grun. In House Creek. Wake Co. P.

Synedra rumpens var. **scotia** Grun. In shallows of pond. Wake Co. CP.

Synedra tabulata (Ag.) Kütz. In rapids of House Creek. Wake Co. P.

Synedra ulna (Nitzsch) Ehrenb. Abundant, cosmopolitan. This species and several of its varieties grow under more diverse conditions than any other of our diatoms. Most abundant in the spring. CP, P, M. (3).

Synedra ulna var. **amphirhynchus** (Ehr.) Grun. See species.

Synedra ulna var. **contracta** Østr. See species.

Synedra ulna var. **danica** (Kütz.) Grun. See species.

Synedra ulna var. **delicatula** Grun. See species.

Synedra ulna var. **longissima** (W. Sm.) Brun. See species.

Synedra ulna var. **oxyrhynchus** (Kütz.) Van Heurck. See species.

Synedra ulna var. **oxyrhynchus** forma **contracta** Hustedt. See species.

Synedra ulna var. **splendens** Kütz. See species.

Asterionella formosa Hassal. Throughout the state in the plankton of ponds, lakes and large streams. Most abundant in the spring. CP, P, M. (3).

Asterionella gracillima (Hantzsch) Heiberg. Abundant in spring and summer, especially in brown waters of the coastal plain. CP.

EUNOTIACEAE

Eunotia alpina (Naeg.) Hustedt. In Limestone Creek. Bladen Co. CP.

Eunotia gracilis (Ehr.) Raben. In the plankton of several ponds. CP.

Eunotia gracilis var. **genuina** Cleve. In plankton of Jones Lake. Bladen Co. CP.

Eunotia lunaris (Ehr.) Grun. In a stream and pool. Onslow and Swain Cos. CP, M. (2).

Eunotia lunaris forma **bilunaris** Mayer. In a swamp pond. CP

Eunotia lunaris var. **subarcuata** (Naeg.) Grun. In swamp off House Creek. Wake Co. P.

Eunotia microcephala Krasske. In rapids of a stream. Swain Co. M. (2).

Eunotia monodon Ehrenb. In House Creek. Wake Co. P.

Eunotia pectinalis (Kütz.) Rabenh. This species and its varieties are abundant in brown-water streams throughout the year, less abundant in summer. Common in sunny seeps and pools in the Piedmont and Coastal Plain in spring. Apparently most abundant in cool, sunny, acid waters. Much rarer in Mountains. CP, P, M. (1).

Eunotia pectinalis var. **biarcuata** A. Bg. Spring seep off House Creek. Wake Co. P.

Eunotia pectinalis var. **gibbosa** A. Bg. Swamp off House Creek. Wake Co. P.

Eunotia pectinalis var. **minor** (Kütz.) Raben. Edge of pond. Pender Co. CP.

Eunotia pectinalis var. **minor** forma **intermedia** Krasske. Spring at 5500 ft. elevation. Swain Co. M. (2).

Eunotia pectinalis var. **ventralis** (Ehr.) Hustedt. Swamp off House Creek. Wake Co. P.

Eunotia robusta Ralfs. Common but not abundant in lakes and streams. CP, P.

Eunotia robusta var. **tetraodon** (Ehr.) Ralfs. In shallows of Lake Mattamuskeet. Hyde Co. CP.

Eunotia sudetica (O. Müll.) Hustedt. Seeps and streams. Caldwell, Onslow and Wake Cos. CP, P, M.

Eunotia tridentula Ehrenb. Creek off Grandfather Mtn. Caldwell Co. M.

Eunotia valida Hustedt. In a spring, Wake Co., creek in Onslow Co. CP, P.

Eunotia veneris (Kütz.) de Toni. Pool at 3000 ft. elevation. Swain Co. M. (2).

Eunotia veneris var. **incisa** (O. Müll.) Hustedt. In a pond Carteret Co., creek in Wayne Co. CP.

Actinella punctata Lewis. Widely distributed in the Coastal Plain, abundant during cooler seasons, especially on *Audouinella violacea*. Collected at 13 stations in the following counties: Bladen, Carteret, Craven, Hoke, Hyde, Onslow, Richmond, Sampson, Scotland, and Wayne. CP.

ACHNANTHACEAE

Achnanthes conspicua A. Mayer. In shallows of Trent River? Craven Co. CP.

Achnanthes lanceolata (Bréb.) Grun. Widely distributed in bottom ooze and on mosses in streams. Rarely abundant. CP, P. M.

Achnanthes lanceolata forma **minor** Schulz. Frequent with the species but less abundant. CP, P, M.

Achnanthes lanceolata var. **elliptica** Cleve. Widely distributed but much less abundant than the species. CP, P, M.

Achnanthes lanceolata var. **rostrata** Hustedt. In the plankton of Trent River. Jones Co. CP.

Achnanthes linearis var. **curta** H. L. Sm. In streams in Wake and Wayne Cos. CP, P.

Achnanthes microcephala Kütz. In a brook. Wake Co. P.

Cocconeis disculus Schum. On moss in Swift Creek. Wake Co. P.

Cocconeis grunovii A. Schmidt. From an unknown locality.

Cocconeis placentula Ehrenb. In creeks. Richmond and Northampton Cos. CP.

Cocconeis placentula var. **euglypta** (Ehr.) Cleve. Attached to vegetation in creeks. Bladen and Craven Cos. CP.

Cocconeis placentula var. **lineata** (Ehr.) Cleve. On algal filaments in pond. Wake Co. P.

Cocconeis scutellum Ehrenb. On mosses in Swift Creek. Wake Co. P.

NAVICULACEAE

Navicula cari var. **angusta** Grun. Bottom of spring at 3000 ft. elevation. Swain Co. M. (2).

Navicula cryptocephala Kütz. Abundant in mats of blue-green algae in streams and and ponds. Bladen, Richmond, Wake Cos. CP, P.

Navicula exigua (Gregory) O. Müll. In a spring. Wake Co. P.

Navicula hungarica var. **capitata** (Ehr.) Cleve. In a creek. Bladen Co. CP.

Navicula minima var. **atomoides** (Grun.) Cleve. In blue-green algal mat, House Creek. Wake Co. P.

Navicula muralis Grun. With the above species. P.

Navicula mutica Kütz. In *Phormidium* mats in two streams. Wake Co. P.

Navicula placenta Ehrenb. Swamps, streams and pools. Swain and Wake Cos. P, M. (2).

Navicula placenta var. **parallela** Krasske. Swamp. Wake Co. P.

Navicula pupula Kütz. In springs and seep. Wake Co. P.

Navicula radiosa Kütz. In creek. Craven Co. CP.

Navicula radiosa var. **tenella** (Bréb.) Cleve. In pool, 3000 ft. elevation. Swain Co. M. (2).

Navicula rostellata Kütz. Abundant, forming brown scum on sandy bottom of Neuse River. Johnston Co. CP. (Identified by C. W. Reimer).

Navicula rhynchocephala Kütz. Widespread in streams. CP, P.

Pinnularia acrosphaeria Bréb. In a marsh. Wake Co. P.

Pinnularia braunii (Grun.) Cleve. In the shallows of a pond. Wake Co. CP.

Pinnularia divergens var. **undulata** Heribaud & Peragallo. In a marsh. Wake Co. P.

Pinnularia gibba Ehrenb. In a pond and seep. Richmond and Wake Cos. CP, P.

Pinnularia latevittata Cleve. In a swamp. Wake Co. P.

Pinnularia legumen Ehrenb. In a spring. Wake Co. P.

Pinnularia macilenta Ehr. emend Cleve. In pool, 3000 ft. elevation. Swain Co. M. (2).

Pinnularia major Kütz. In bottom ooze of marshy areas. Pitt, Swain and Wake Cos. CP, P, M. (2).

Pinnularia mesolepta (Ehr.) W. Smith. In creek. Pasquotank Co. CP.

Pinnularia viridis (Nitzsch) Ehrenb. In spring seep. Wake Co. P.

Pinnularia viridis var. **sudetica** (Hilse) Hustedt. In swamp. Craven Co. CP.

Neidium affine (Ehr.) Pfitz. In ooze of ditch and creek. Craven and Wayne Cos. CP. (Identified by C. W. Reimer)

Neidium iridis (Ehr.) Cleve. In ooze of ponds and streams. Carteret, Wake and Wayne Cos. CP.

Neidium iridis var. **vernalis** (Ehr.) Cleve. In ooze of still water. Craven, Pitt and Wake Cos. CP, P.

Neidium ladogensis var. **densistriata** (Østr.) Foged. In muck of creek and lake. Bladen and Wayne Cos. CP.

Diploneis domblittensis (Grun.) Cleve. In bottom of roadside canal. Hyde Co. CP.

Diploneis elliptica (Kütz.) Cleve. In bottom ooze of spring and pool. Hyde and Onslow Cos. CP.

Stauroneis anceps forma **linearis** Cleve. In rapids of House Creek, Wake Co. P.

Stauroneis crucicula (Grun.) Boyer. In two creeks. Hertford and Northampton Cos. CP.

Stauroneis phoenicenteron Ehr. In pool, 3000 ft. elevation. Swain Co. M. (2).

Amphipleura pellucida Kütz. In brackish water of Cape Fear River south of Wilmington. New Hanover Co. CP.

Frustulia crassinervia Bréb. In rapids of two creeks. Halifax and Wake Cos. CP, P.

Frustulia rhomboides (Ehr.) de Toni. Widespread in streams and shallows of ponds. Rarely abundant. CP, P, M.

Frustulia vulgaris Thwaites. Widespread in streams and shallows of ponds. Rarely abundant. CP, P.

Gyrosigma obtusatum (Sulliv.) Boyer. On rocks in rapids of Haw Rver. Chatham Co. P.

Amphiprora costata Hustedt. Infrequent in brook. Bladen Co. CP.

Amphiprora ornata Bailey. Rare in plankton of Lake Johnson. Wake Co. P.

Vanheurckia rhomboides var. **crassinervia** (Bréb.) Van Heurck. In pool, 3000 ft. elevation. Swain Co. M. (2).

Vanheurckia vulgaris (Thw.) Van Heurck. In pool, 3000 ft. elevation. Swain Co. M. (2).

GOMPHONEMATACEAE

Gomphonema acuminatum Ehrenb. In Bridger's Creek. Martin Co. CP.

Gomphonema angustatum var. **producta** Grun. In two creeks. CP, M.

Gomphonema augur Ehrenb. Common to abundant in one pond and stream. CP, P. (1).

Gomphonema constrictum Ehrenb. On rocks and mosses in brook. Wake Co. P.

Gomphonema constrictum var. capitata (Ehr.) Cleve. In two brooks. CP, P.

Gomphonema constrictum var. cuneata A. Schmidt. In shallows of a pond. Warren Co. CP.

Gomphonema dichotomum Kütz. Common, attached to filamentous green algae in spring. CP, P.

Gomphonema gracile Ehrenb. In spring and swamp of House Creek. Wake Co. P.

Gomphonema lanceolatum Ehrenb. In shallows of several ponds and brooks. Wake Co. P.

Gomphonema parvulum Kütz. On mosses and stones in rapids of streams. CP, P.

Gomphonema parvulum var. subelliptica Cleve. In rapids of House Creek. P.

Gomphonema subclavatum Grun. In two creeks. Bertie and Duplin Cos. CP.

CYMBELLACEAE

Cymbella affinis Kütz. From unknown locality.

Cymbella aspersa (Ehr.) Cleve. In spring off House Creek. Wake Co. P.

Cymbella ehrenbergii Kütz. With the above species. Also in mountain pool. Wake and Swain Cos. P, M. (2).

Cymbella gracilis (Rabh.) Cleve. In rapids of several streams and shallows of ponds. CP, P.

Cymbella hauckii Van Heurck. Mountain pool, 3000 ft. elevation. Swain Co. M. (2).

Cymbella lanceolata (Ehr.) Van Heurck. In a spring. Wake Co. P.

Cymbella mexicana Ehrenb. In shallows of Trent River. Craven Co. CP.

Cymbella naviculiformis Auersw. In pool at Bradley Fork. Swain Co. M. (2).

Cymbella norvegica Grun. Ooze in a brook. Johnston Co. CP.

Cymbella obtusiuscula (Kütz.) Grun. In marsh. Wake Co. P.

Cymbella scotica W. Sm. In a pool at Bradley Fork. Swain Co. M. (2).

Cymbella tumida (Bréb.) Van Heurck. Widespread. CP, P.

Cymbella turgida (Gregory) Cleve. On moss in creek. Wake Co. P.

Cymbella ventricosa Kütz. Widespread in ponds and streams. CP, P.

Amphora ovalis Kütz. One spring and a pool. Dare and Wake Cos. CP, P.

Epithemia zebra var saxonica (Kütz.) Grun. In a creek. Caldwell Co. M.

Rhopalodia gibba var. ventricosa (Ehr.) Grun. In two creeks. CP, P.

Rhopalodia gibberula (Ehr.) O. Müll. In a spring. Wake Co. P.

NITZSCHIACEAE

Bacillaria paradoxa Gmelin. In vegetation and ooze in springs and streams during cool seasons, except in the mountains. A temperature above 20°C seems limiting. CP, P. M. (1).

Nitzschia acicularis W. Smith. In tufts of blue-green algae, House Creek. Wake Co. P.

Nitzschia communis Rabenh. In seep on a brick wall, Fort Macon. Carteret Co. CP.

Nitzschia dissipata (Kütz.) Grun. Widespread in brooks and creek. CP, P.

Nitzscnia hennedyi W. Sm. Seep in sandy road. Carteret Co. CP.

Nitzschia ignorata Krasske. In plankton of black-water pond. Carteret Co. CP.

Nitzschia obtusa var. scalpelliformis Grun. In a brook and pond. Bladen and Richmond Cos. CP.

Nitzschia palea (Kütz.) W. Smith. In several streams and ponds. CP.

Nitzschia praetexta Ehrenb. In seep in sandy road. Carteret Co. CP.

Nitzschia sigmoidea (Ehr.) W. Smith. From an unknown locality.

Nitzschia thermalis var. minor Hilse. In brook. Bladen Co. CP.

Nitzschia vermicularis (Kütz.) Grun. In creek. Craven Co. CP.

SURIRELLACEAE

Cymatopleura solea (Bréb.) W. Smith. In brook, Snead's Ferry. Pender Co. CP.

Surirella angusta Kütz. In two streams. CP, P.

Surirella arctissima A. Sm. In shallows of Singletary Lake. Bladen Co. CP.

Surirella biseriata var. bifrons forma amphioxys (W. Smith) Hustedt. In several streams. CP, P.

Surirella elegans Ehrenb. In marshy canal. Pitt Co. CP.

Surirella linearis var. constricta (Ehr.) Grun. In pond. Carteret Co. CP.

Surirella ovata Kütz. In brook, Snead's Ferry. Bladen Co. CP.

Surirella robusta var. **splendida** (Ehr.) van Heurck. Rare in three ponds. CP, P. (1).

Surirella tenera var. **nervosa** A. Schmidt. In Swift Creek. Wake Co. P.

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THE DEVELOPMENT OF THE OVULE AND MEGAGAMETOPHYTE IN THE GENUS *ILEX* L.[1]

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Ilex L., the largest of the three genera which compose Aquifoliaceae DC. with over four hundred species, is widely distributed in both the temperate and tropical zones. The remaining two genera, *Phelline* Loes. with ten species and the monotypic *Nemopanthus* Raf., are geographically restricted, the former to New Caledonia, the latter to northeastern North America. Loesner (1901) excludes from the family the genus *Sphenostemon* Baill. The anatomical data collected by Pennington (1953) certainly warrants this exclusion.

The investigation here reported treats of six species in the principal subgenera of *Ilex*, namely *Euilex* Loes. and *Prinos* (L.) Loes. Various morphological features indicate that these species display the principal variations in generic expression and, therefore, are appropriate for an investigation into the problem of *Ilex* embryology.

The Problem

In his monograph of Aquifoliaceae, which stands pre-eminent among taxonomic considerations of the family, Loesner (1901, 1908) proposes an intricate system of classification based chiefly on gross morphological features. Loesner's treatment has been twice revised (1919, 1942). In the last study cited, *Ilex* L. is divided into the following five subgenera: *Rybonia* Loes., with one species; *Byronia* (Endl.) Loes., with twenty species; *Yrbonia* Loes., with one species; *Euilex* Loes., with over four hundred species; and *Prinos* (L.) Loes, with sixteen species.

There has been some disagreement with Loesner's systematic treatment particularly with reference to his choice of criteria in making sectional and subsectional distinctions. Loesner alone, however, has given consideration to all Aquifoliaceae, and his systematic treatment in its final form culminates fifty years of investigation into the taxonomic problems of this family (chart I). The fact that the subgeneric taxa show certain distinctions but yet appear to merge and overlap in most of their features may suggest that the family is actively differentiating.

The entire order Celastrales has been neglected with regard to embryological studies. Schürhoff (1921) in the only detailed embryological account from Aquifoliaceae treats primarily megagametogenesis in *I. aquifolium* L. with some attention to pollen tube formation and to the early stages of endosperm development. A comparative study on the embryology of *Ilex* made by Mauritzon (1936) and confined to the structure of the ovule in four species is based entirely on material from herbarium specimens and, therefore, limited to gross features. In a later account, Mauritzon (1939) compares many of these features with those in other Celastralean families.

Some attention has been given to the embryological features of Celastraceae. Jönsson (1879/80) includes in his detailed study of several angiospermous families a treatment of megagametogenesis and ovule development in *Euonymus latifolius* Mill. Considerable information with regard to megagametogenesis in ten species from the subfamilies Celastroideae Loes. and Tripterygioideae is presented by Andersson (1931). Both of these investigations are considered valuable for comparison with the observations reported here.

To define the pattern of megagametogenesis typical for *Ilex* has been the primary objective of this investigation, and, therefore, the study has been extended to a group of species which together characterize the generic expression. All features of development have been considered for their possible taxonomic significance within the genus and for their value in clarifying the relationship of Aquifoliaceae to Celastraceae.

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CHART I

Family Aquifoliaceae DC

Tribe I Ilicеae Dumort.
Genus *Ilex* L.
Subgenus I Rybonia Loes.
Subgenus II Byronia (Endl.) Loes.
Series A. Eubyronia Loes.
Series B. Micrococca Loes.
Subgenus III Yrbonia Loes.
Subgenus IV Euilex Loes.
Series A. Lioprinus Loes. (4 Sections)
Section 2. Cassinoides Loes.
I. opaca Ait.
Series B. Paltoria (Ruiz. & Pav.) Maxim. (4 sections)
Section 2. Polyphyllae Loes.
I. crenata Thunbg.
I. crenata forma *conveza* (Makino) Rehd
Series C. Aquifolium (Tournef.) Maxim. (11 sections)
Section 2. Aquifolioides Loes. (2 subsec-sections)
Subsection a. Oxyodontae Loes.
I. cornuta Lindl. & Paxt.
I. cornuta forma *Burfordii* (De France) Rehd.
Section 3. Microdontae Loes. (5 subsec-tions)
Subsection c. Vomitoriae Loes.
I. vomitoria Ait.
Series D. Thyrsoprinus Loes. (5 sections)
Subgenus V Prinos (L.) Loes.
Series A. Euprinus Loes.
I. verticillata (L.) Gray
Series B. Prinoides Gray
I. decidua Walt.
Genus *Nemopanthus* Raf.
Tribe II Phellineae Loes.
Genus *Phelline* Labill.

Materials and Methods

The collection of flowers of each species studied was extended over a period of several years. In order fully to ascertain the complete sequence of megagametogenesis, these collections were started at the onset of flower initiation in March or April and were continued through May. Material used in this study is represented by the following vouchers deposited in the University of North Carolina Herbarium: *Ilex opaca* Ait. (Charlottesville, Va., *Herr 10*; Lexington, Va., *Herr 14*; Chapel Hill, N. C., *Herr 16*) *I. crenata* Thunbg. (Chapel Hill, N. C., *Herr 60*) *I. crenata* forma *conveza* (Makino) Rehd. (Charlottesville, Va., *Herr 2*; Lexington, Va., *Herr 11*; Chapel Hill, N. C., *Herr 20*; Pinehurst, N. C., *Herr 56*) *I. cornuta* Lindl. & Paxt. (Pinehurst, N. C., *Herr 36*) *I. cornuta* forma *Burfondii* (De France) Rehd. (Lexington, Va., *Herr 12*; Chapel Hill, N. C., *Herr 22*) *I. vomitoria* Ait. (Afton, Va., *Herr 15*; Chapel Hill, N. C., *Herr 19, 47, & 57*; Long Beach, N. C., *Herr 25*; Orton Plantation, N. C., *Herr 26*) *I. verticillata* (L.) Gray (Charlottesville, Va., *Herr 9*; Chapel Hill, N. C., *Herr 43*) *I. decidua* Walt. (Charlottesville, Va., *Herr 8*; Chapel Hill, N. C., *Herr 29, 30, 31 & 45*)

Adequate fixation of the material was achieved with several fluids, but FPA 50 (formalin-propionic acid-50% ethyl alcohol) generally proved most suitable for all stages of development. The mature megagametophyte is usually best preserved by Allen's modification of Bouin's fluid. All fluids used were prepared as recommended by Johansen (1940). Fixation was usually performed in the field except where the extraction of ovules proved necessary.

All material prepared for sectioning was dehydrated by the tertiary butyl alcohol method and embedded in paraffin as recommended by Johansen (1940). Longitudinal sections were prepared from whole ovularies or from individual ovules. Sections 8–10 μ proved most useful for stages in megasporogenesis and early megagametogenesis. The later stages of megagametogenesis were best shown by sections 12–15 μ in thickness.

At the beginning of the study, adequate staining was achieved by the application of safranin and fast green as recommended by Johansen (1940). Later this technique was altered to achieve better differentiation between the cytoplasm and cell walls. The sections were mordanted for thirty minutes as suggested for Johansen's alcoholic hematoxylin technique and were then stained in safranin to which a few drops of alcoholic hematoxylin were added.

The magnification of each photograph and camara lucida drawing is cited in the legends.

Observations and Discussion

The continuous series of events in the development of the megagametophyte in *Ilex* has been divided here into three major phases. Each phase is described in accordance with the observations from the six species studied. The order in which the species are considered follows their sequence in Loesner's treatment of the genus (chart I).

The Development of the Sporogenous Tissue.— The initial steps in the development of the ovule follow a similar pattern in all species studied.

The ovular protuberance arises at the apex of the loculus on either the axile or parietal surface of the loculus. Uniform elongation soon extends the protuberance toward the base of the loculus. Subsequently, the apex of the lengthened structure undergoes uneven growth and curves, in most instances, toward the central axis of the ovulary. The appearance of the rudimentary, single integument and nucellus coincides with the curvature of the young ovule. As growth continues in all parts of the ovule, the sporogenous tissue is differentiated within the nucellus.

The primary archesporial cell has not been observed in *I. opaca*. In the nucellus of young ovules, there generally occurs a group of three sporogenous cells. These conspicuous cells are readily distinguishable from the nucellus by their larger size, denser cytoplasm, and more prominent nuclei. The massive nucleolus is the most conspicuous feature distinguishing the sporogenous nucleus from the nuclei of the nucellar cells. The frequent occurrence of the three sporogenous cells in a linear sequence suggests their origin from a single, hypodermal archesporial cell (fig. 22). Several preparations indicate that any one of the three cells may enlarge to become the megaspore mother cell. Alteration of the linear sequence may be a direct consequence of growth in the functional sporogenous cell or may indicate that the cells are independent in origin (fig. 23). The nonfunctional cells often appear elongate and are compressed against the adjacent nucellus. Little growth of the nucellus accompanies the formation of sporogenous cells. The production of several sporogenous cells is regarded as typical in this species, but the absence of nonfunctional sporogenous tissue in some preparations indicates that the primary archesporial cell may enlarge directly becoming the megaspore mother cell.

Differentiation of the sporogenous tissue is accompanied by distinct enlargement of the ovule localized in the funiculus and integument. Pronounced curvature in the funiculus early establishes the anatropous form. The single integument develops uniformly around the entire circumference of the ovule, and its length finally equals that of the nucellar mass. Prominent on the inner face of the funiculus is a globular protuberance situated immediately above the inner portion of the integument. Rudimentary second ovules often appear collateral to the first at the alternate point of ovule origin (fig. 1).

In *I. crenata*, the primary archesporial cell originates in the hypodermal nucellus. It often divides before it undergoes marked enlargement to produce two sporogenous cells (fig. 39). The division spindle, which is distinctly larger than that found in adjacent cells (fig. 39, cf. fig. 40), is oriented in essentially the horizontal plane. Consequently, the two resultant sporogenous cells are more or less in the same plane (fig. 41). That these two cells have originated from a single archesporium is further indicated by their similarity and close association clearly evident in cross sections of the young ovule (fig. 42). Infrequent and probably of minor importance is the formation of three or more sporogenous cells from a single archesporium (fig. 43). The sporogenous tissue consisting of two cells is considered typical for this species, but the primary archesporial cell may enlarge directly to become the megaspore mother cell (fig. 44). The cell apparently originates in the hypodermis and subsequently assumes a deeper position as a result of periclinal divisions in the epidermis. Periclinal division figures have not been observed in the epidermis, but their occurrence is inferred from the size and shape of some of the epidermal cells. It is evident that the crassinucellate condition becomes established early in this species.

Initial curvature of the young ovule immediately precedes the formation of the archesporial cell (fig. 4). The development of the sporogenous tissue is accompanied by a marked increase in the size of the ovule and continued curvature to establish the typical anatropous form. As in *I. opaca*, the integument extends slightly beyond the nucellus and shows uniform growth. The funiculus usually contains a distinct provascular strand but seldom shows the prominent protuberance on its inner surface. In most instances, the locule considerably exceeds the ovule in size (fig. 5).

The archesporial cell of *I. cornuta* originates in the hypodermis of the nucellus (fig. 58). It may become a megaspore mother cell by direct enlargement or may produce a sporogenous tissue of two or three cells. Each division may occur in the horizontal plane to produce three sporogenous cells in linear sequence (fig. 59). The three sporogenous cells occur in a T-shaped arrangement when the upper cell of the two-celled stage divides in the vertical plane (fig. 60). In the latter instance, the large, basal cell is clearly the functional one. Rarely, the sporogenous tissue becomes multicellular with up to nine cells dis-

tributed over several consecutive sections (fig. 61).

During the development of the sporogenous tissue, the ovule closely resembles those observed in *I. opaca*. Curvature of the funiculus establishes a similar degree of anatropy. The inner surface shows a prominent funicular protuberance, and the integument usually extends beyond the nucellus. In most instances, the loculus is considerably larger than the ovule (fig. 10).

The archesporium and sporogenous tissue has not been observed in *I. vomitoria*.

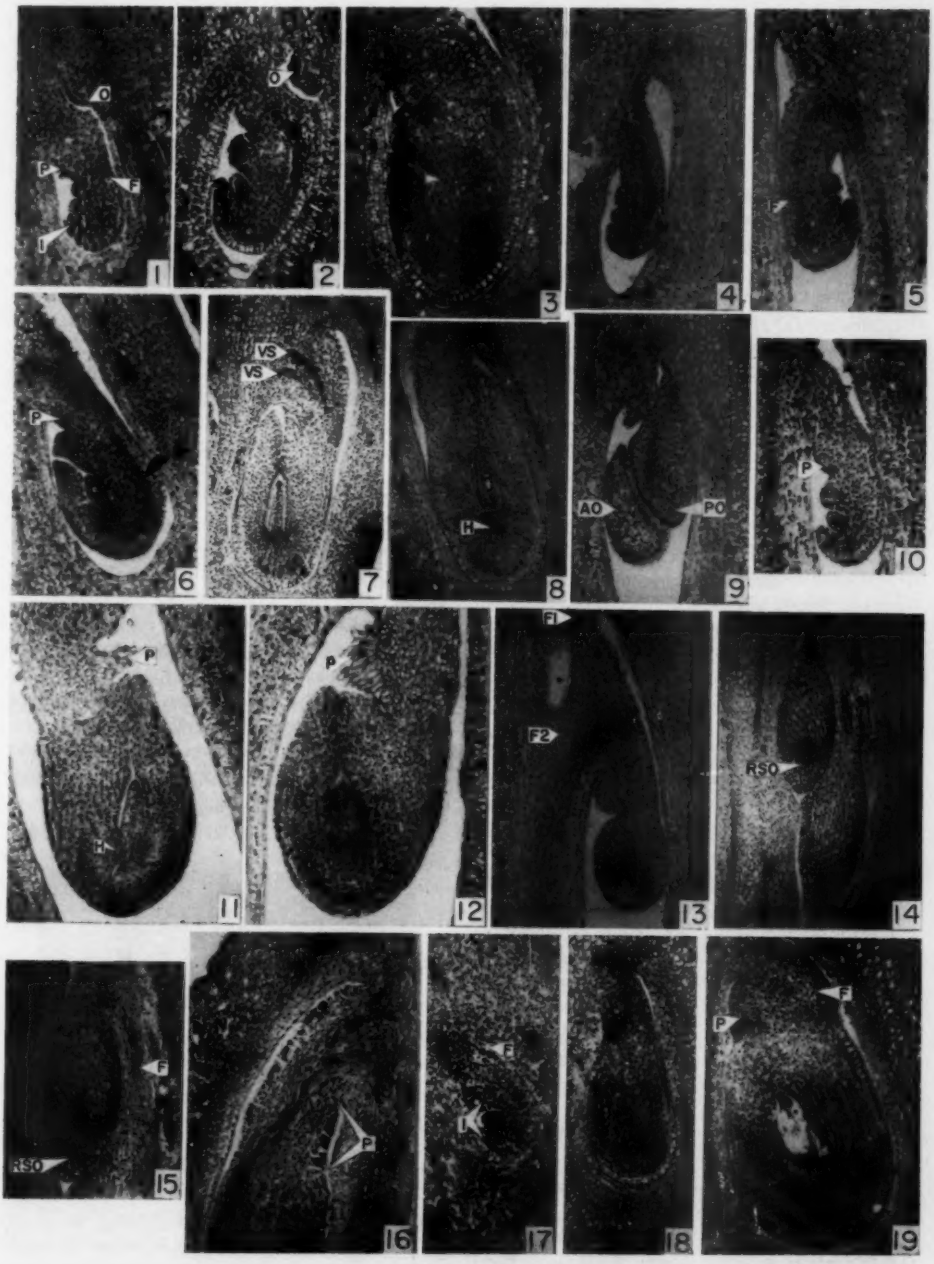
In *I. verticillata*, a hypodermal archesporium may become the megaspore mother cell directly or may produce two or more sporogenous cells. A sporogenous tissue of two cells is the usual condition. The cells may occur in vertical series, or two or three of them may occur in a horizontal plane (fig. 75). Early extensive growth of the nucellus may bring the sporogenous cells to considerable depth in the nucellar mass (fig. 76).

As in *I. opaca*, the ovule completely fills the loculus and through curvature acquires the typical anatropous form. The integument is a massive structure and extends beyond the nucellus. Elongation of the funiculus is considerably less than in the species already mentioned (fig. 17).

The development of sporogenous tissue in *I. decidua* has not been observed.

There are certain inherent difficulties in a study of this kind which somewhat limit the accurate evaluation of variable developmental features. The principal hindrance lies in the necessary use of complex techniques which make unfeasible the frequent observation of each phase of development. Therefore, the frequency of occurrence and significance of variations in the developmental pattern cannot be conclusively determined. Thus, an attempt will be made here and in later discussion merely to indicate the

FIGS. 1–3. *Ilex opaca* Ait. Fig. 1. Sporogenous tissue differentiation; ovule shows initial curvature of funiculus (F); integument (I) equals nucellus in length; protuberance (P) present; rudimentary second ovule (O) collateral to the first. ×100. Fig. 2. Early megasporogenesis; anatropous form emphasized; marked growth in outer portion of integument restricts width of micropyle; enlargement evident in cells of chalazal epidermis; rudimentary second ovule (O) enlarges; loculus and ovule nearly equal in size. ×100. Fig. 3. Close of megasporogenesis; growth of integument extensive in outer portion; differentiation in chalazal epidermis marked by intensely stained cell walls and nuclei. ×100. FIGS. 4–9. *Ilex crenata* Thunbg. Fig. 4. Sporogenous tissue differentiation; initial curvature of ovule; integument equals nucellus in length; funicular protuberance absent at this stage. ×100. Fig. 5. Sporogenous tissue differentiation; curvature of funiculus (F) establishes anatropous form; integument elongates uniformly and extends beyond nucellus; funicular protuberance still absent; loculus exceeds ovule in size. ×100. Fig. 6. Early megasporogenesis; differentiation in chalazal epidermis; funicular protuberance (P) develops. ×100. Fig. 7. Close of megasporogenesis; marked elongation of integument and nucellus; funicular protuberance small, compressed by integumentary growth; two provascular strands (VS) evident in funiculus; differentiation in epidermis progressively increased. ×67. Fig. 8. Megagametogenesis; ovule enlargement uniform in all parts; hypostase (H) clearly evident. ×67. Fig. 9. Simultaneous development in parietal (PO) and axile (AO) ovules. ×100. FIGS. 10–13. *Ilex cornuta* Lindl. and Paxt. Fig. 10. Sporogenous tissue differentiation; anatropous form indicated; funicular protuberance (P) prominent; integument extends beyond nucellus; hypostase (H) evident; loculus much larger than ovule. ×100. Fig. 11. Megasporogenesis; ovule enlarges and assumes anatropous form; integument elongates markedly and is characteristically tapered; funicular protuberance (P) modified by radial elongation of peripheral cells; differentiation evident in chalazal epidermis, but cells remain small; loculus much larger than ovule. ×100. Fig. 12. Megagametogenesis; funicular protuberance (P) enlarges by radial extension of peripheral cells; loculus much larger than ovule. ×100. Fig. 13. Suggestion of dual origin of ovule; base of funiculus is thicker at its upper (F1) and lower (F2) extremities than at its center. ×100. FIGS. 14–16. *Ilex vomitoria* Ait. Fig. 14. Funicular protuberance develops into rudimentary second ovule (RSO); funiculus of first ovule lengthens. ×53. Fig. 15. Higher magnification clarifies point of origin of rudimentary second ovule (TSO); funiculus (F) of first ovule elongate and narrow. ×67. Fig. 16. Funicular protuberance (P) characteristically large in this species; two vascular strands enter funiculus. ×100. FIGS. 17–19. *Ilex verticillata* (L.) Gray Fig. 17. Sporogenous tissue differentiation; ovule fills loculus; integument (I) extends beyond nucellus; funiculus (F) short; anatropous form indicated. ×100. Fig. 18. Megasporogenesis; anatropous position attained; funicular protuberance prominent; differentiation evident in chalazal epidermis. ×100. Fig. 19. Megagametogenesis; ovule enlargement uniform; funiculus (F) short; protuberance (P) directly contacts apex of integument; differentiation in epidermis as previously noted. ×100.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FIGS. 1–19

variable features and to emphasize their coincident appearance in different species.

General features in the development of sporogenous tissue which are clearly evident in each of the species studied may represent the typical generic expression. The archesporium consistently originates in the hypodermal nucellus, as it does in most of the Angiosperms, and demonstrates two principal variations in behavior: it may undergo direct enlargement to become the megaspore mother cell, or it may divide to form several sporogenous cells. Minor variations of this general pattern appear in each species.

Similarity between certain species is quite evident with regard to specific details in the development of sporogenous tissue. Frequently in *I. opaca* and *I. cornuta* three sporogenous cells are produced in linear sequence. In both of these species, early growth of the nucellus is slight. A two-celled sporogenous tissue is prevalent in both *I. crenata* and *I. verticillata*, and growth of the nucellus early establishes the crassinucellate condition.

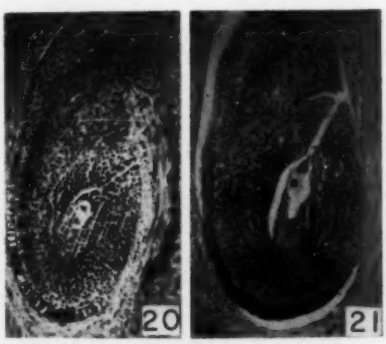
The sporogenous tissue usually originates by equal cell divisions, and any one of the several cells which compose the tissue may become the megaspore mother cell. One instance to the contrary appears in *I. cornuta* where the three sporogenous cells occur in a T-shaped arrangement. The general configuration of the three cells indicates that the functional sporogenous cell was clearly designated by the unequal transverse division of the archesporium. The small upper cell by vertical division produces two cells which resemble the parietal cells or cover cells (*deckzelle*) frequently found among Angiosperms.

The development of the ovule is markedly similar in all of the species studied. Quite evident at this stage is the unitegmic condition and at least an indication of the anatropous form. The principal variable features with regard to ovule structure are the reduced funiculus in *I. verticillata* and the absence of the funicular protuberance in *I. crenata*. In *I. cornuta* and to a less extent in *I. crenata*, the ovule is distinctly smaller than the loculus. This condition merely indicates that the enlargement of the ovulary is proportionally greater than that of the ovule.

Many of the structural features associated with the formation of sporogenous tissue have been described previously in other species of *Ilex* and also in some members of Celastraceae. Schürhoff (1921) reports that the hypodermal archesporium in *I. aquifolium* originates before the formation of the single integument, and that it enlarges directly to become the megaspore mother cell. There is no reference in this account either to the division of the archesporium or to the existence of two or more sporogenous cells. Mauritzon (1936) in his study of four species of *Ilex* notes certain "marks" on the single integument which disclose its formation by the coalescence of two integuments. This phenomenon supports derivation of *Ilex* from the bitegmic Celastraceae, a proposal treated further in later discussion. Evidence of fusion of two integuments is, however, absent in the species studied here. If the single integument originated by the fusion of two structures, as well might have been the case, then the coalescence has been sufficiently complete to erase the visible evidence for its occurrence.

Several observations reported by Andersson (1931) in his study of two subfamilies in the Celastraceae correspond to certain of those noted in *Ilex*. As a general occurrence, the hypodermal archesporium may either become the megaspore mother cell directly or divide to produce several sporogenous cells. The "two archesporial cells" reported to occur in the same horizontal plane in *Celastrus scandens* L. corresponds to the two-celled sporogenous tissue of *I. crenata* and *I. verticillata*. In this same species of *Celastrus*, the appearance of two parietal cells above the megaspore mother cell provides a configuration very similar to the T-shaped arrangement of sporogenous cells previously noted here in *I. cornuta*.

The evident similarity between *Ilex* and



FIGS. 20–21. *Ilex decidua* Walt. Fig. 20. Early megagametogenesis; ovule enlargement uniform; granular material distinct in chalazal epidermis. ×100. Fig. 21. Close of megagametogenesis; micropylar passage wide; funicular protuberance prominent and distinct; funiculus short as in *I. verticillata*. ×100.

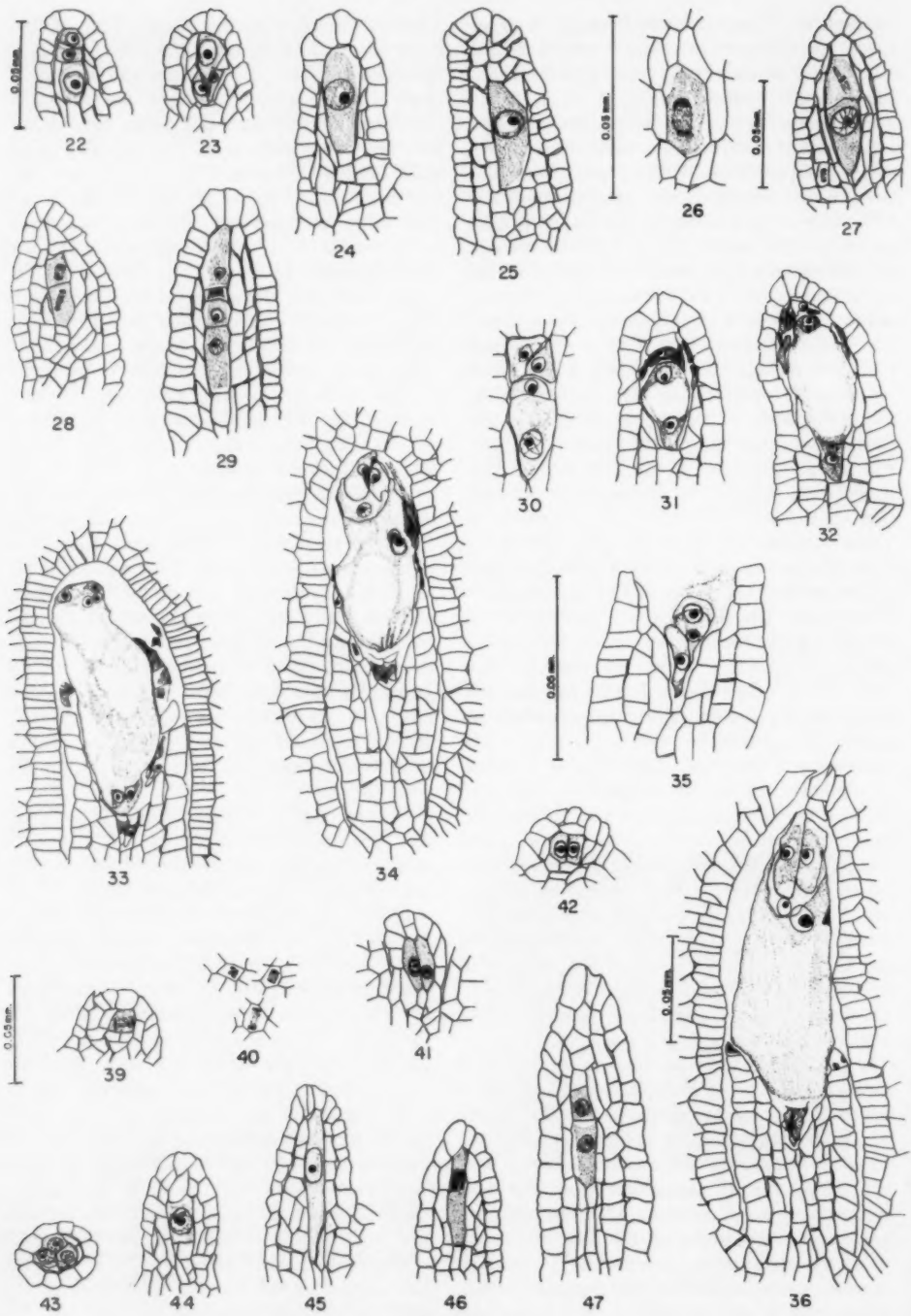
Celastrus in sporogenous tissue formation is one of several developmental features recorded in this study which indicate relationship between Aquifoliaceae and Celastraceae.

Megasporogenesis.—In *I. opaca*, marked elongation of the functional sporogenous cell completely obliterates the nonfunctional tissue. The nucellus has enlarged, but growth coincident with the formation of the hypodermal megaspore mother cell has resulted from anticlinal division and elongation of the epidermal cells. That the sporogenous cell does not considerably increase in width is disclosed by the absence of disintegration in the lateral nucellus (fig. 24). Subsequent growth of the nucellus occurs in part as a result of periclinal divisions in the epidermal cells. Frequently three or four layers of cells are produced above the megaspore mother cell, and cell enlargement is evident throughout the nucellus (fig. 25). The crassinucellate condition is thus established and is followed by meiosis I. Variation in the size of the dividing mother cell indicates that meiosis I may occur before maximal cell enlargement is achieved. The division figure is distinctly larger than that observed in the cells of the nucellus (fig. 26, cf. fig. 27), integument, and funiculus. At late telophase, a transverse wall is formed to establish the two daughter cells. Unequal division rather than secondary enlargement accounts for the larger size of the chalazal cell. The two cells together conform generally to the size of the megaspore mother cell. Meiosis II closely follows the first division and may or may not be synchronized in the two nuclei (fig. 27, 28). The formation of transverse walls between the daughter nuclei of meiosis II produces four megaspores in linear arrangement. Diversity with regard to the location of the spores in the nucellus indicates that the rate of nucellar growth is variable. Three layers of nucellus may surround the megaspores. In many instances, however, the micropylar spore occurs immediately below the epidermis. The functional megaspore is larger than the other three and usually occupies the chalazal position. The degree of disintegration may be similar in each of the three micropylar spores or may distinctly vary (fig. 29). Frequently the second micropylar spore disintegrates well in advance of the first and third spores, and persistence of the first micropylar spore has occasionally been noted. The chalazal megaspore often exhibits highly vacuolated cytoplasm prior to nuclear division. Only rarely does it show marked enlargement (fig. 30).

Several changes in the development of the ovule are quite evident at the onset of megasporogenesis. The nucellus undergoes decided enlargement, and curvature of the funiculus continues. The integument extends beyond the nucellus, and growth is more pronounced in its outer portion. The cells of the outer layer in the chalazal region of the ovule are distinctly larger than other integumentary cells. This increase in size is the first of several changes which produce a characteristic pattern of differentiation in the ovule epidermis. Elongation of the funiculus is usually restricted to the unequal growth effective in curving the ovule. The protuberance shows little change and is situated close to the inner portion of the integument. Frequently the provascular strand is quite apparent in the funiculus. In many instances, the rudimentary second ovule enlarges and distorts the funiculus (fig. 2).

Throughout megasporogenesis, ovule enlargement results chiefly from integument growth. The outer portion of the integument, which continues to grow more rapidly than the region adjacent to the funiculus, encloses the nucellus and restricts the width of the micropylar passage. Differentiation in the outer cell layer is evident in the chalazal region of the ovule and along the adjacent margin of the raphe. In addition to their larger size, the cells in this region show intensely stained cell walls and nuclei. The cytoplasmic stain is less apparent here than in the surrounding areas (fig. 3).

Continued growth of the nucellus in *I. crenata* may produce several layers of cells above the megaspore mother cell. Frequently, however, the cell occupies a hypodermal position (fig. 45). It may appear quite narrow and elongate. During meiosis I (fig. 46), periclinal divisions are sometimes evident in the nucellar epidermis and account for the frequent occurrence of the daughter cells beneath three layers of nucellus. The daughter cells are distinctly slender but exhibit the form and behavior of those of *I. opaca* (fig. 47). Meiosis II has not been observed, but the four megaspores are produced in linear or T-shaped sequence beneath one to several layers of nucellus (fig. 48). Differentiation of secondary sporogenous tissue may occur in the nucellus produced above the megaspores. In one instance, two cells appear which simulate the typical sporogenous characteristics, but their origin and fate are not known (fig. 49). Their position suggests an independent origin for each cell. The functional megaspore occupies the usual chalazal



FIGS. 22–36, 39–47

position. Disintegration of the three micropylar spores shows the same diversity described previously. As in *I. opaca*, vacuolation of the cytoplasm precedes nuclear division in the functional megaspore.

During the early stages of megasporogenesis, the ovule enlarges and nearly fills the loculus. The integument elongates chiefly in its outer portion thus to enclose the nucellus and to restrict the micropylar passage.

Especially noticeable at this stage is the late appearance of the funicular protuberance above the inner portion of the integument. The outer layer of cells in the chalazal region is similar in appearance to that of *I. opaca* (fig. 6).

At the close of megasporogenesis, the ovule shows a marked increase in length effected for the most part by elongation of the integument and nucellus. The extensive growth of the integument is uniform around the circumference of the ovule, and the micropylar passage, therefore, is lengthened considerably. In some instances, the inner portion of the integument contacts and somewhat distorts the funicular protuberance. Differentiation continues in the epidermis at the chalaza but is less evident here than in the comparable stage for *I. opaca* (fig. 7).

In *I. cornuta*, growth of the nucellus concurrent with megasporogenesis follows the general pattern observed in *I. opaca*. Enlarged megaspore mother cells, therefore, may be hypodermal (fig. 62) or may occur beneath two to four layers of nucellar tissue (fig. 63). Meiosis and the formation of the daughter cells has not been observed in this species, but the megaspores occur in a linear sequence and show the same characteristics previously noted (fig. 64).

One preparation shows the unusual occurrence of a hypodermal megaspore mother cell adjacent to four megaspores. A single cell which simulates sporogenous characteristics occurs in the basal nucellus. These diverse structures, evident in two consecutive sections, occur in an ovule which shows other features typical of ovule development at the four megaspore stage (fig. 65).

Throughout megasporogenesis, the ovule enlarges and assumes the typical anatropous form. The integument undergoes marked, uniform elongation to extend far beyond the nucellus, and its apex is characteristically tapered. In many instances, the micropylar passage is longer than the entire nucellus. The funicular protuberance becomes quite modified in appearance by the radial elongation of some of its peripheral cells. Marks of differentiation in the outer cell layer are noted in the chalazal region. These changes, however, are not accompanied by a marked increase in the size of the cells. The ovule is still considerably smaller than the loculus (fig. 11).

The hypodermal megaspore mother cell of *I.*

FIGS. 22–36. *Ilex opaca* Ait. Fig. 22. Three sporogenous cells in linear sequence. ×333. Fig. 23. Functional sporogenous cell enlarges to displace and compress nonfunctional sporogenous cells. ×333. Fig 24. Hypodermal megaspore mother cell. ×333. Fig. 25. Megaspore mother cell beneath four layers of nucellus. ×333. Fig. 26. Megaspore mother cell at telophase of meiosis I. ×567. Fig. 27. Meiosis II advanced to metaphase in micropylar daughter cell; nucleus of chalazal daughter cell at early prophase. ×333. Fig. 28. Meiosis II synchronized at telophase in both daughter cell nuclei. ×333. Fig. 29. Four megaspores in linear sequence; chalazal spore functional; disintegration advanced in second, micropylar spore. ×333. Fig. 30. Four megaspores in linear arrangement; functional, chalazal spore develops vacuolate cytoplasm prior to nuclear division. ×333. Fig. 31. Two-nucleate phase prior to marked enlargement; central vacuole separates two nuclei; disintegration evident in nonfunctional megaspores and in some nucellus. ×333. Fig. 32. Four-nucleate phase; large central vacuole maintained; nucellar disintegration increasingly evident in micropylar region. ×333. Fig. 33. Eight-nulceate phase; two antipodal cells formed early; their separation from free nuclei results from fixation; disintegration of micropylar nucellus complete. ×333. Fig. 34. Mature megagametophyte; synergids show slight disintegration; two polar nuclei in typical peripheral location near pollen tube fragments; antipodal cells distorted. ×333. Fig. 35. Enlarged, vacuolate antipodal cells. ×567. Fig. 36. Mature megagametophyte; synergids enlarged; polar nuclei fused; antipodal cells distorted; nucellus present only at chalazal region. ×333.

FIGS. 39–47. *Ilex crenata* Thunbg. Fig. 39. Division of archsporial cell nucleus prior to marked cell enlargement. ×333. Fig. 40. Division figures in young nucellus. ×333. Fig. 41. Two sporogenous cells oriented in same horizontal plane. ×333. Fig. 42. Cross-section of nucellus; close association of two sporogenous cells suggests their origin from single archesporium. ×333. Fig. 43. Cross-section of nucellus; three sporogenous cells from single archesporium. ×333. Fig. 44. Enlarging archesporium. ×333. Fig. 45. Elongate, hypodermal megaspore mother cell. ×333. Fig. 46. Megaspore mother cell at metaphase of meiosis I ×333. Fig. 47. Daughter cells of meiosis I. ×333.

vomitoria is similar in most respects to those observed in *I. opaca* and *I. cornuta* (fig. 69). Information with regard to the nature of meiosis or the formation of the daughter cells is incomplete. The occurrence of the megaspores in linear sequence beneath one to three layers of cells indicates that extension of the nucellus for the most part follows megasporogenesis. With regard to their form and behavior, the megaspores conform to the typical pattern previously described (fig. 70).

In the ovule, development follows the same general pattern observed in *I. opaca*. The ovule is usually smaller but exhibits proportionally a larger funicular protuberance.

Enlargement of the functional sporogenous cell in *I. verticillata* to form the megaspore mother cell is associated with marked growth of the nucellus. The enlarged cell usually occurs beneath two or three layers of nucellus tissue (fig. 77). The megaspore mother cell is consistently slender and elongate. It does not, however, conform to the extreme slender form observed in some cells of *I. crenata*. Meiosis I occurs as described previously and is accompanied by nucellar growth. The daughter cells, usually found beneath several layers of nucellus (fig. 78), show size variation in different preparations—an indication of their enlargement prior to meiosis II. The second meiotic division has not been observed. In the typical manner, the four megaspores are produced in linear sequence with the functional spore in the chalazal position (fig. 79). The frequent occurrence of daughter cells and megaspores beneath several layers of cells indicates the growth of the nucellus is continuous throughout megasporogenesis. The appearance of two sets of megaspores in a single ovule suggests that two sporogenous cells may function simultaneously (fig. 80). Evidence with regard to the disintegration pattern shown by the non-functional spores is not available.

The initial steps in megasporogensis are accompanied by marked growth of the ovule. Ovules containing the megaspore mother cell have fully assumed the anatropous form. The integument extends well beyond the nucellus and encloses it thus sharply restricting the micropylar passage. At this stage, the funicular protuberance is not a prominent structure. Differentiation in the ovule epidermis resembles the pattern described for *I. opaca* at a similar stage of development (fig. 18).

The megaspore mother cell of *I. decidua* occurs beneath a single layer of nucellus, and apparently it never achieves the size usually attained in the other species (fig. 88). Meiosis and the daughter cells have not been observed. The megaspores show the same form and behavior observed in the other species. Elongation of the nucellus occurs, but additional cells are not produced above the megaspores (fig. 89).

At the close of megasporogenesis, the ovule is similar in all respects to those of *I. verticillata*.

Megasporogenesis in *Ilex* discloses the general features found prevalent among the majority of Angiosperms. Quite consistently the megaspores are produced in a linear sequence, and the functional spore occupies the chalazal position. The principal variable feature associated with this phase of development is the growth rate of the nucellus which may fluctuate even within the same species. It has been previously noted that the crassinucellate condition in *I. crenata* may be early established as disclosed by the appearance of sporogenous tissue beneath several layers of nucellus. However, the fact that the megaspore mother cell in many instances is hypodermal indicates that the early growth of the nucellus may be very slight. The appearance of hypodermal megaspores in *I. vomitoria* and *I. decidua* indicates that the delay of nucellar extension may even prevail throughout megasporogenesis. Growth of the nucellus occurs throughout the entire mass, but its apical extension results primarily from periclinal division of the epidermal cells.

In some instances, as noted in *I. crenata*, marked extension of the nucellus above the megaspores is followed by the secondary differentiation of sporogenous cells. The fate of these sporogenous cells has not been determined. However, the unusual anomaly in *I. cornuta*, which discloses the simultaneous occurrence of a megaspore mother cell and four megaspores in the hypodermis of an elongate nucellar mass, may have originated with the formation of secondary sporogenous cells. This latter instance, in addition to the simultaneous occurrence of two sets of megaspores in *I. verticillata*, clearly indicates that the functional capacity is not confined to a single cell in the sporogenous tissue.

There is general uniformity with regard to the size and shape of the cells involved in megasporogenesis. The very slender megaspore mother cells of *I. crenata* and *I. verticillata* provide the principal exceptions and, for their prevalence, represent a characteristic expression in these species. From its general occurrence, the reduced

megaspore mother cell may be considered characteristic for *I. decidua*.

The variable features in the generally uniform pattern of ovule development in some instances characterize certain species. In *I. cornuta*, the funicular protuberance is distinguished by the radial extension of its peripheral cells. Also quite distinctive in this species is the epidermal layer of the ovule which is composed of cells with small dimensions.

The massive funicular protuberance of *I. vomitoria* is proportionally larger than those of other species and conversely the reduced size of this protuberance is distinctive for *I. crenata*.

Each of the species in the subgenus *Euilex* discloses at least slight variation in the form and appearance of the ovule. On the other hand, the ovules of *I. decidua* and *I. verticillata* in the subgenus *Prinos* are markedly similar. The short funiculus and reduced funicular protuberance are the principal characteristic features.

Megasporogenesis in *I. aquifolium* studies by Schürhoff (1921) conforms to the pattern for *Ilex* described here. The position of the megaspore mother cell within the nucellus is not disclosed, but the megaspores are produced in linear sequence with the functional spore consistently in the chalazal position.

Members of Celastraceae studied by Andersson (1931) are similar to *Ilex* in the arrangement and behavior of the megaspores. Usually the mother cell and megaspores are hypodermal. In *Celastrus scandens*, however, the megaspore mother cell may appear beneath three to four layers of nucellus. In general, the extent of nucellar growth is slightly greater in *Ilex* than in the members of Celastraceae. The general similarity in the crassinucellate condition of the two groups, however, supports previously recorded evidence of their close relationship.

Megagametogenesis.—Nuclear division in the functional megaspore of *I. opaca* produces the two-nucleate phase in megagametogenesis. The nuclei are at first confined to the center of the more or less vacuolate cell, and usually at this stage few changes are evident in the disintegrating, nonfunctional megaspores. Growth of the two-nucleate phase is probably directly related to the formation and enlargement of a central vacuole which separates the nuclei and displaces them toward the micropylar and chalazal extremities of the cell. Subsequent to their separation, the nuclei become situated in dense cytoplasm, and the nonfunctional megaspores and adjacent nucellus begin to show evidence of marked disintegration (fig. 31). The formation of the central vacuole has not been observed in *I. opaca*, but observations from several species suggest that, as will be described for *I. crenata*, it arises by drift and coalescence of other vacuoles. Continued growth of the two-nucleate megagametophyte compresses the nonfunctional megaspores against the nucellus. At maximal enlargement, disintegration is clearly shown in the lateral nucellus and is often initiated in the cell layers above the megaspores.

The smallest expression of the four-nucleate megagametophyte consistently exceeds the maximal size attained by the two-nucleate phase. The large central vacuole is maintained, and the two pairs of nuclei are situated in the micropylar and the chalazal regions. Disintegration of the nucellus is slightly more apparent than previously noted (fig. 32). Continued elongation of the megagametophyte is accompanied by extensive disintegration in the nucellus. At its maximal size, the four-nucleate phase contacts only fragmentary nucellus in the micropylar region. Disintegration is slight or decidedly evident in all lateral nucellus except the epidermis near the chalazal region.

Synchronized nuclear division produces four nuclei at the micropylar and at the chalazal region of the megagametophyte. The central vacuole is frequently replaced by sparsely vacuolate cytoplasm. The eight-nucleate phase is rather transitory, and maturation frequently precedes further enlargement with the early formation of two or three antipodal cells. Each nucleus is centrally located in the antipodal cell and is surrounded by dense cytoplasm. The separation of the free chalazal nuclei from those enclosed is apparently a fixation phenomenon (fig. 33). Extensive disintegration has occurred in the lateral nucellus, and the micropylar region of the megagametophyte directly contacts the integument.

Maturation continues with the migration of a single nucleus from each quartet toward the central region of the megagametophyte. The remaining free nuclei are enclosed by cell membranes establishing the egg and two synergids in the micropylar region and three chalazal antipodal cells. Features which distinguish the egg from the synergids are its larger size and less vacuolate cytoplasm. Disintegration of the synergid cells may occur prior to syngamy. In some instances, however, these cells persist through the early stages of endosperm formation.

Frequently the polar nuclei are situated at the periphery of the megagametophyte, and adjacent, darkly stained structures are considered fragments of the pollen tube (fig. 34). Distortion of the antipodal cells closely follows their formation, and the cells frequently persist in this condition through the early stages of endosperm formation. Occasionally they enlarge and develop highly vacuolate cytoplasm (fig. 35). Separation of the antipodal cells from the rest of the megagametophyte is considered a fixation phenomenon. Disintegration continues in the marginal cells until only the chalazal region of the megagametophyte remains enclosed by nucellar tissue. Slight enlargement of the megagametophyte subsequent to its maturation is frequently accompanied by growth of the egg and synergids. Fusion of the polar nuclei often occurs before the pollen tube enters the ovule. The position of the diploid or triploid nucleus proximal to the egg probably results from elongation of the egg cell (fig. 36). Close association between the egg and sperm nuclei is frequently observed, but the simultaneous occurrence of two sperm near the egg nucleus is rare (fig. 37). The sperm usually appear ellipsoidal, but they may elongate and coil. Close association is occasionally observed between the sperm and the diploid secondary nucleus. The sperm, however, appears more distinct when it occurs in the surrounding cytoplasm (fig. 38). Ultimate fusion of the sperm and the diploid secondary nucleus gives rise to the triploid, primary endosperm nucleus.

FIGS. 37–38. *Ilex opaca* Ait. Fig. 37. Enlarged egg (E); two sperm (Sp) in close association with egg nucleus; pollen tube (PT) adjacent to egg. ×525. Fig. 38. Sperm cell (Sp) to right of large, secondary nucleus (SN); chromatin of sperm intensely stained; cytoplasm barely discernible. ×525.

Ovule development during megagametogenesis is marked chiefly by uniform growth in all regions. Differentiation in the outer layer of cells becomes more apparent and is extended over a wider area.

The two-nucleate megagametophyte in *I. crenata* is very similar to that of *I. opaca*. In the early stages of its development, the nuclei are closely associated in the center of the vacuolate cytoplasm. There usually occur two or three layers of nucellus above the disintegrating spores. Separation of the nuclei is accompanied by the movement of the lateral vacuoles to the center of the megagametophyte (fig. 50, 51). Subsequent elongation of the cell is not as marked here as in *I. opaca*. When maximal size is attained, however, the nuclei are situated at the micropylar and chalazal extremities separated by a central vacuole (fig. 52). Slight disintegration of the nucellus is evident.

Division of the nuclei usually occurs in the horizontal plane to give rise to the four-nucleate phase. One or both pair of nuclei may become separated from the extremities of the megagametophyte by small vacuoles. Occasionally abundant nucellus is produced above the megagametophyte and three or four cells there may display sporogenous characteristics (fig. 53). During continued growth, the nuclei are pressed to the extremities and become reoriented from the horizontal to a vertical position (fig. 54).

The atypical pattern of development shown in figure 55 was seemingly initiated by a simultaneous advancement of two megaspores to the two-nucleate phase. Each pair of nuclei in these separate cells behaved as dyads of a single four-nucleate megagametophyte. The nuclei in the upper cell (ref. fig. 55) moved toward the micropyle while those of the lower cell moved toward the chalaza. This phenomenon is here reported because of the important bearing it may have on studies of nuclear movement in the megagametophyte of Angiosperms.

Reorientation of the nuclei in the four-nucleate phase is closely followed by division. The appearance of megagametophytes with four micropylar nuclei and only two chalazal nuclei indicates that the divisions may not be synchronized (fig. 56). Nucellus disintegration at the eight-nucleate stage follows the same pattern observed in *I. opaca*. Furthermore, the two species exhibit marked similarity with regard to maturation of the megagametophyte (fig. 57).

During the development of the two- and four-nucleate phases, the ovule undergoes considerable enlargement. A comparison with earlier stages shows that growth is uniform and proportional in all parts. The epidermis in the chalazal region usually discloses little change in its general appearance (fig. 8; cf. fig. 7).

In the chalazal region, a large group of cells develop thick cell walls to become the hypostase, a tissue frequently found among Angiosperms and present in all species of *Ilex* studied here. In *Ilex* the hypostase is confined to the chalaza, but in some groups it extends into the basal nucellus. In the absence of information regarding its formation and function, further discussion of the hypostase will be deferred. However, this tissue has been pointed out in several photographs in which it is clearly visible.

Megagametogenesis in *I. cornuta* follows a pattern very similar to that of *I. opaca*. The nucellus in which the two-nucleate phase occurs is shorter and broader than those observed in the other species (fig. 66). Marked growth of the megagametophyte occurs during the two-nucleate phase. The four-nucleate phase undergoes slight enlargement which causes the disintegration of the micropylar nucellus. Division in the horizontal plane of one nucleus from each pair is simultaneous with vertical division in the other two nuclei. This phenomenon clarifies the three-dimensional aspect of nuclear organization in the eight-nucleate megagametophyte (fig. 67).

The occurrence of six-nucleate stages serves as evidence for the occasional absence of synchronous division of the four nuclei. In most instances, the six-nucleate condition shows four nuclei at the chalazal region. The enclosure of two of these nuclei by cell membranes early establishes two antipodal cells.

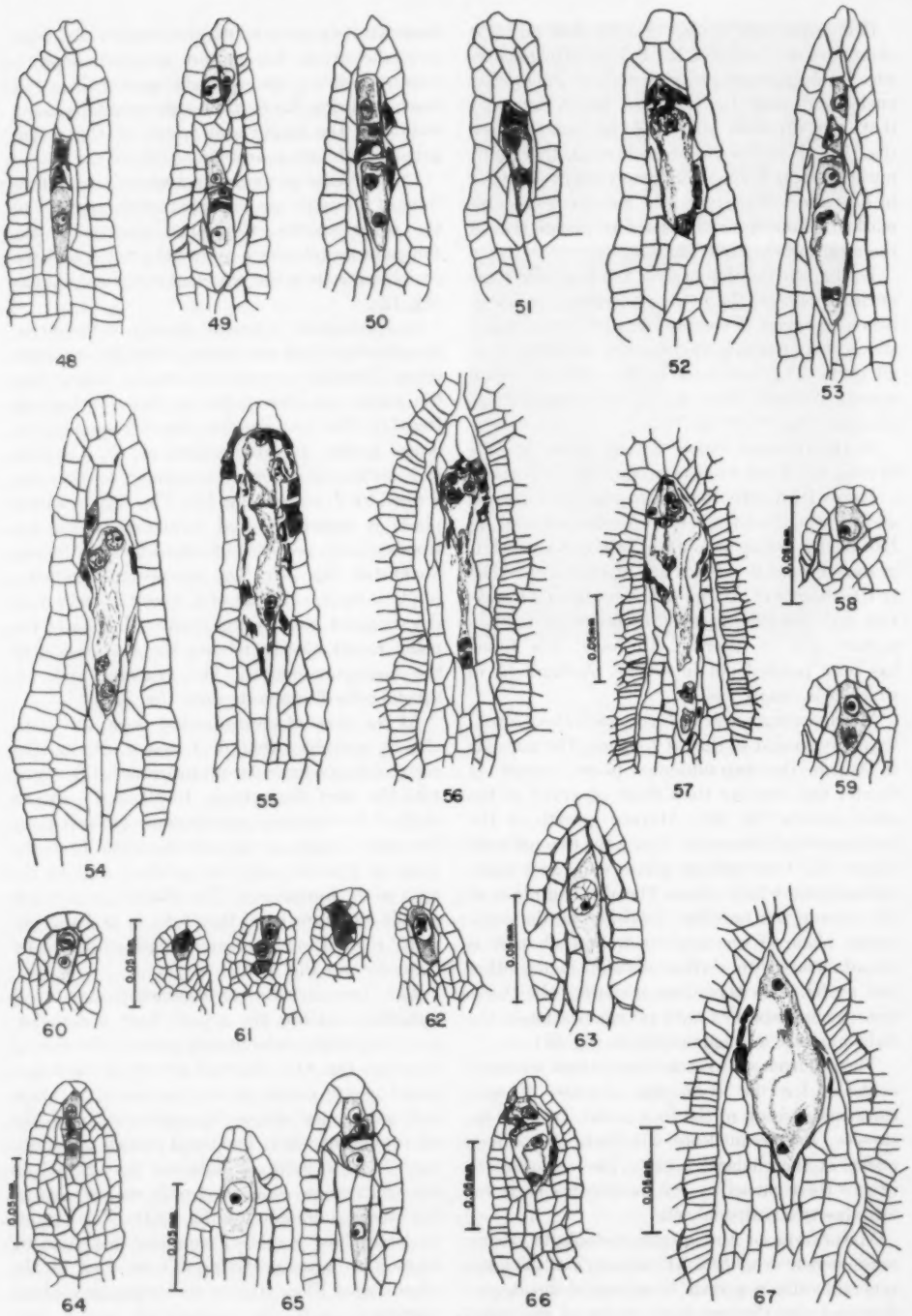
Maturation of the megagametophyte occurs as previously described. All preparations show the synergid cells in a state of advanced disintegration and also disclose early fusion of the polar nuclei. The antipodal cells characteristically demonstrate greater width than length and show early distortion. The mature megagametophyte does not attain a size markedly greater than that reached during the four-nucleate stage. In many instances, the length and width of the megagametophyte are nearly equal (fig. 68).

In the course of megagametogenesis, modifications of the ovule are less apparent than in any of the other species. Continued growth of the funicular protuberance by radial extension of the peripheral cells is the principal structural change (fig. 12).

In *I. vomitoria*, extensive growth of the megagametophyte does not occur in the two-nucleate phase. Distinct vacuoles are usually present and the nuclei are surrounded by dense cytoplasm (fig. 71). The four-nucleate phase frequently enlarges to the dimensions attained in *I. opaca*, and nuclear orientation is similar to that described for *I. crenata* (fig. 72). The eight-nucleate phase is transitory, and formation of the antipodals may precede differentiation of the egg apparatus (fig. 73). The mature megagametophyte is similar to that of *I. opaca* in pattern of development but is often smaller. Fusion of the polar nuclei closely follows the maturation of the megagametophyte. The synergid and antipodal cells disintegrate early (fig. 74).

At the close of megagametogenesis, the ovule closely resembles that of *I. opaca*. Its smaller size and larger funicular protuberance still constitute the chief distinctions. In all of the species studied, the funicular protuberance extends along the entire length of the free funiculus from the point of junction with the ovulary wall to the apex of the integument. The distinct prominence of this structure here, therefore, is probably directly related to the greater length exhibited by the funiculus (fig. 16).

The two-nucleate megagametophyte of *I. verticillata* exhibits the typical form and undergoes only slight enlargement prior to division of its nuclei (fig. 81). Maximal growth of the megagametophyte occurs at the four-nucleate phase and sometimes effects the obliteration of the micropylar nucellus. The usual persistence of one layer of cells, however, indicates that growth in the epidermis is concurrent with enlargement of the megagametophyte (fig. 82). The eight-nucleate phase is seldom seen, and distortion by fixation is more pronounced here then in the other stages. Elongation of the megagametophyte continues, and its micropylar region frequently contacts only fragmentary nucellus



FIGS. 48–67

(fig. 83). The mature phase conforms to the pattern of organization previously described. In many instances, the antipodal cells are distorted early, but they persist through the early stages of endosperm development. The nucellus which encloses the chalazal region of the megagametophyte is less conspicuous here than in the other species (fig. 84). Frequently the synergid cells grow extensively and persist throughout the early stages of endosperm development.

The mature megagametophyte exhibits one important, uniform feature consistently observed in every species studied but heretofore not described. All preparations show the mature phase so oriented that the egg forms toward the side of the ovule next to the funiculus and raphe, and the two synergids form toward the opposite side from which the micropyle opens. This characteristic orientation diagrammatically shown in figure 87 is especially evident in longitudinal sections which pass simultaneously through the raphe and the micropyle. Such a median longitudinal section is here shown for *I. verticillata* (fig. 85, 86).

Megagametogenesis is accompanied by an increase in the size of the ovule. The funiculus is relatively short, and the protuberance usually directly contacts the apex of the integument. Differentiation in the epidermis follows the pattern described for *I. opaca* (fig. 19).

The two-nucleate megagametophyte and nonfunctional megaspores of *I. decidua* usually occur beneath a single layer of nucellus (fig. 90). Slight enlargement of the megagametophyte is evident prior to nuclear division. The four-nucleate phase usually contacts only nucellar fragments in the micropylar region (fig. 91). Megagametophytes have not been observed at the eight-nucleate phase, but maturation occurs in the typical manner. In some instances, the synergid cells exhibit marked distortion. Frequently, however, they enlarge to dimensions never attained in the other species studied. The cells exceed the length reached by the egg and usually contain conspicuous vacuoles (fig. 92; cf. fig. 93).

The development of the two-nucleate phase is accompanied by enlargement of the ovule and continued differentiation in the outer layer of cells. Granular cytoplasm associated with differentiation in the epidermal layer is more clearly evident here than in the other species (fig. 20).

At the close of megagametogenesis, the ovule closely resembles those of *I. verticillata*. The micropylar passage occasionally appears quite wide (fig. 21).

Megagametogenesis in *Ilex* conforms very closely to the *Polygonum* type and follows a rather uniform pattern in the various species studied. Disintegration of the nucellus in the

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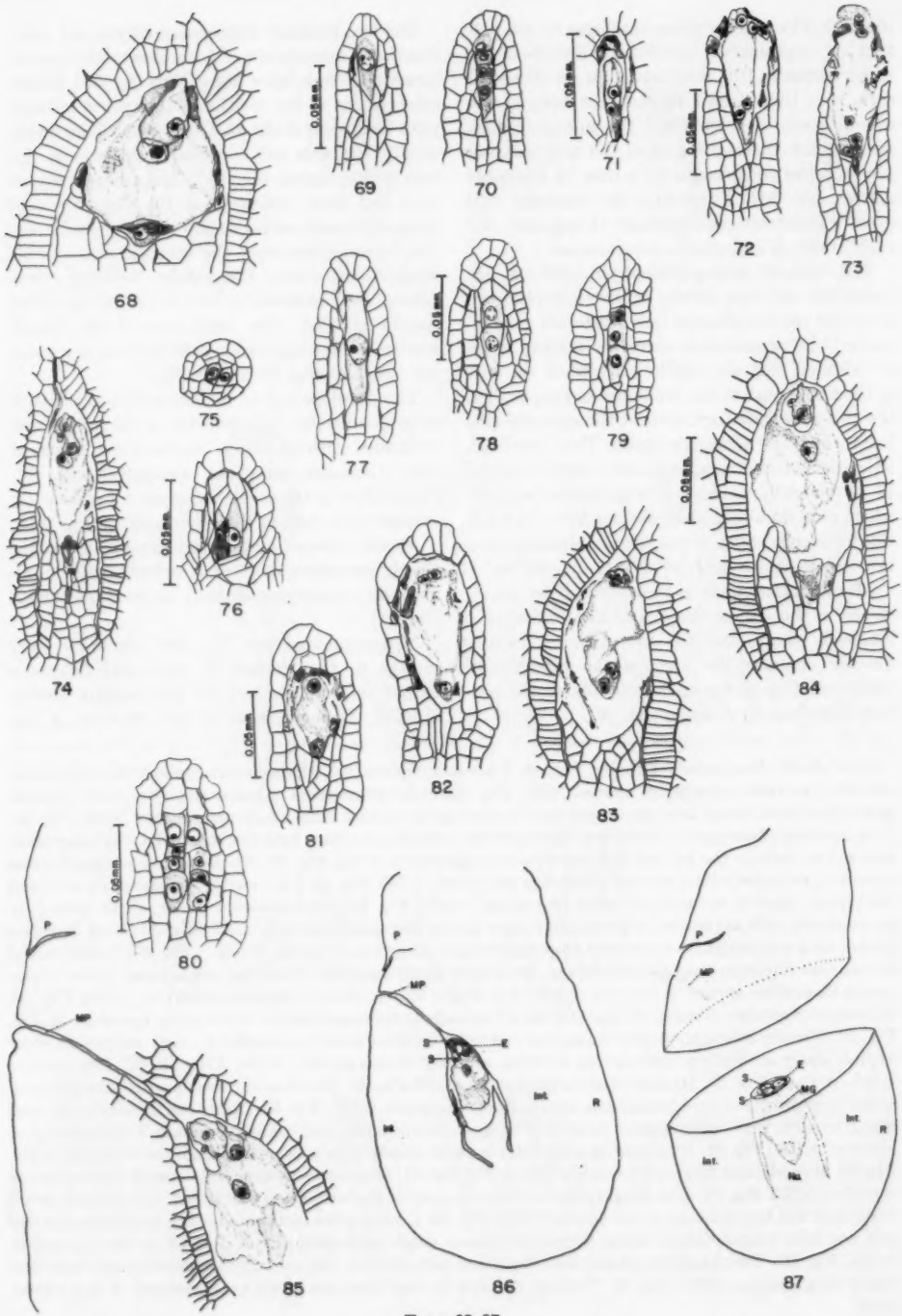
FIGS. 48–57. *Ilex crenata* Thunbg. Fig. 48. Four megaspores in T-shaped arrangement; disintegration advanced in three micropylar spores. ×333. Fig. 49. Four megaspores in linear sequence with chalazal spore functional; secondary sporogenous cells develop in nucellar mass above megaspores. ×333. Fig. 50. Two-nucleate phase; prior to marked enlargement; vacuoles in center between the nuclei; disintegration scarcely evident in the 1st and 2nd micropylar megaspores. ×333. Fig. 51. Nuclei of two-nucleate phase separate; vacuoles collect centrally between the nuclei. ×333. Fig. 52. Two-nucleate phase at its maximal size; large, central vacuole separates two nuclei. ×333. Fig. 53. Four-nucleate phase; three secondary sporogenous cells appear in large nucellar mass above megagametophyte. ×333. Fig. 54. Four-nucleate phase; each nuclear phase reoriented from a horizontal to a vertical plane. ×333. Fig. 55. Paired nuclei in two two-nucleate megagametophytes, developed simultaneously from two megaspores, move in opposite directions as would the two dyads of a single four-nucleate megagametophyte. ×333. Fig. 56. Six-nucleate phase; division of chalazal nuclei retarded; disintegration in micropylar nucellus. × 333. Fig. 57. Mature megagametophyte; micropylar polar nucleus partly concealed by egg; antipodals show typical early distortion; micropylar nucellus entirely disintegrated. ×333. FIGS. 58–67. *Ilex cornuta* Lindl. & Paxt. Fig. 58. Hypodermal archesporium. ×333. Fig. 59. Division of lower sporogenous cell indicates formation of three sporogenous cells in linear sequence. ×333. Fig. 60. Upper cell divides in vertical plane to form three sporogenous cells in T-shaped arrangement; section plane makes T-shaped aspect obscure. ×333. Fig. 61. Nine sporogenous cells in three consecutive sections through the nucellus. ×267. Fig. 62. Hypodermal megaspore mother cell. ×267. Fig. 63. Megaspore mother cell beneath four layers of nucellus. ×333. Fig. 64. Four megaspores in linear sequence; chalazal spore functional; disintegration advanced in 2nd and 3rd micropylar spores. ×267. Fig. 65. Consecutive sections showing megaspore mother cell and four megaspores at same horizontal plane; single sporogenous cell present in basal nucellus. ×333. Fig. 66. Two-nucleate phase; disintegration advanced in the micropylar nucellus and nonfunctional megaspores. ×267. Fig. 67. Nuclear division in four-nucleate phase synchronized at metaphase. ×333.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FIGS. 68–87

micropylar region, which is initiated by growth of the four-nucleate phase, is one of the principal uniform features. Nucellus, therefore, is absent in the micropylar region of the mature megagametophyte. The polar nuclei fuse early in all of the species studied, and the antipodal cells early assume a distorted appearance. Throughout the genus, there is a frequent persistence of the synergids and distorted antipodal cells until the early stages of endosperm formation. The phenomenon of megagametophyte orientation within the anatropous ovule here described for its importance as a uniform feature in *Ilex* (ref. fig. 87) has received little attention in embryological studies of other Angiosperms.

The separation of the antipodal cells from the rest of the megagametophyte, frequently noted in this study, has been reported for many Angiosperm species. Hewitt (1939) from his study of *Lobelia amoena* considers this phenomenon a natural occurrence. Its occurrence in *Ilex*, on the contrary, appears to be a direct consequence of uneven shrinkage of the megagametophyte during fixation.

Slight enlargement of the antipodal cells noted in *I. opaca* occurs infrequently and is a phenomenon entirely dissimilar in degree to the unusual growth of these cells in certain other Angiosperms (cf. Osterwalder, 1898 and Wyatt, 1955). The size increase does, however, disclose the slight tendency toward the retention of a primitive condition, i.e. the formation of a well developed chalazal nutritive tissue.

Considerable variation has been noted throughout *Ilex* with regard to the growth rate of the megagametophyte at its various phases of development. Further observation perhaps would reveal greater uniformity and would probably show that size variation is an environmental response.

Evidence recorded here for *I. crenata* and collected also from other species clearly indicates that nuclear separation in the two-nucleate phase is related to the coalescence of vacuoles in the center of the megagametophyte. Flint and Johansen (1958) report that in *Fritillaria* certain "radiations" formed directly or indirectly by extruded nucleolar material either hold the nuclei in place or direct their movement. That vacuoles have a part in the movement of nuclei is no longer tenable in the view of these workers. In consideration of many studies, including the well documented account by Flint and Johansen, the author suggests that more than one mechanism is operative in the movement of nuclei in megagametophytes of Angiosperms.

The rather frequent occurrence of the six-nucleate condition suggests the absence of synchronization in the nuclear divisions which give rise to the eight-nucleate phase. The location of four of the six nuclei at either the micropylar or chalazal end shows that division in the four-

FIG. 68. *Ilex cornuta* Lindl. & Paxt. Fig. 68. Mature megagametophyte showing characteristic reduced length; synergids advanced in disintegration; polar nuclei fused; antipodal cells typically broader than long. ×333. FIGS. 69–74. *Ilex vomitoria* Ait. Fig. 69. Hypodermal megaspore mother cell. ×267. Fig. 70. Four megaspores in linear sequence. ×267. Fig. 71. Two-nucleate phase; migration and coalescence of vacuoles in central region. ×333. Fig. 72. Four-nucleate phase; shift of nuclear pairs from horizontal to vertical plane; micropylar nucellus in marked disintegration. ×267. Fig. 73. Eight-nucleate phase; antipodals formed early; fragmentary nucellus in micropylar region. ×267. Fig. 74. Mature megagametophyte; polar nuclei fused; egg enlarged; pollen tube extends from micropylar passage. ×267. FIGS. 75–86. *Ilex verticillata* (L.) Gray. Fig. 75. Cross-section of nucellus; two sporogenous cells from a single archesporium. ×267. Fig. 76. Three sporogenous cells more or less at same horizontal plane, deep in nucellar mass. ×333. Fig. 77. Elongate megaspore mother cell beneath two layers of nucellus. ×333. Fig. 78. Daughter cells of Meiosis I beneath four cell layers of nucellus ×267. Fig. 79. Four megaspores in linear sequence; disintegration advanced in the 1st and 2nd micropylar spores is scarcely evident in the 3rd spore. ×267. Fig. 80. Two sets of megaspores in linear arrangement. ×333. Fig. 81. Two-nucleate phase; nuclei at extremities of cell separated by vacuoles. ×267. Fig. 82. Four-nucleate phase. ×267. Fig. 83. Eight-nucleate phase; fragmentary nucellus in the micropylar region. ×267. Fig. 84. Mature megagametophyte; polar nuclei fused; synergids enlarged. ×267. Fig. 85. Partial outline of ovule to show funicular protuberance (P), micropylar passage (MP) and the mature megagametophyte. ×267. Fig. 86. Outline of ovule (cf. fig. 85) to show funicular protuberance (P), micropylar passage (MP), integument (Int), raphe (R), synergids (S) and egg (E). ×267. FIG. 87. *Ilex* L. fig. 87. Diagrammatic outline of typical *Ilex* ovule appropriately sectioned to show position of the egg (E) and synergids (S) with relation to other ovule parts labelled as in fig. 86 except for nucellus (Nu) and megagametophyte (MG). c. ×267.

nucleate phase may be retarded in either pair of nuclei.

Variations in the form of the ovule noted during megasporogenesis are retained throughout further development. For the most part, few modifications are introduced through the course of megagametogenesis, and development is marked chiefly by ovule enlargement. To this generalization, *I. cornuta* presents the only exception. The absence of extensive growth in this species accounts for the distinctive appearance of the ovules.

The development of the megagametophyte in *I. aquifolium* follows the same pattern described here. According to Schürhoff (1921), the polar nuclei usually fuse before fertilization, and the antipodal cells are little developed.

Mauritzon (1936) mentions that the nucellus in *Ilex* is destroyed by growth of the four-nucleate phase. Apparently this information was derived from *I. aquifolium*, since the material from the other species treated (*I. amara*, *I. montana*, and *I. nitida*) was collected from herbarium specimens.

There is marked similarity between *Ilex* and members of Celastraceae with regard to megagametogenesis. In the species studied by Andersson (1931), nucellar disintegration is more evident during the early phases than it is in *Ilex*. The micropylar portion of the four-nucleate phase in *Tripterygium wilfordii* Hook f. usually directly contacts the inner integument. In *Celastrus scandens*, the nuclei of the four-nucleate phase disclose division figures similar in orientation to those observed in *I. cornuta*. The early appearance of antipodal cells in *T. wilfordii* suggests that the eight-nucleate phase is a transitory phase. In all species studied by Andersson, the nucellus which encloses the chalazal end of the mature megagametophyte is less conspicuous than in *Ilex*. Quite generally, the synergids enlarge and become vacuolate, and in *T. wilfordii* these cells elongate markedly to appear very similar to those observed in *I. decidua*. Also of general occurrence is the distinct integumentary tapetum.

With regard to megagametogenesis in *Euonymus latifolius*, the study by Andersson verifies the observations of Jönsson (1879/80).

Throughout the course of megagametogenesis, the nature of nucellar growth in both the Celastraceae and Aquifoliaceae is somewhat clarified. It is quite evident from Andersson's study that both the crassinucellate and tenuinucellate conditions are expressed in Celastraceae. The crassinucellate condition is prevalent in *Ilex* but is usually expressed rather mildly. Only in a few instances has extensive growth of the nucellus accompanied megagametogenesis. A tendency toward the tenuinucellate condition is evident in *I. decidua* as disclosed by the hypodermal position of the nonfunctional spores associated with the two-nucleate phase. This tendency was actually indicated earlier in both *I. vomitoria* and *I. decidua* by the hypodermal position of the four megaspores.

Thus, the total evidence available indicates that the crassinucellate condition in *Ilex*, although mildly expressed, is indeed more firmly established than in Celastraceae. This feature may, therefore, prove quite significant, since it indicates a derived position for Celastraceae.

PHYLOGENETIC CONSIDERATIONS

In all species studied, the position of the ovule may be either parietal or axile with regard to its point of origin. Origin from the axile locus is the usual condition, but many preparations which show this typical situation also disclose a small, rudimentary ovule in the alternate parietal location (fig. 1). The parietal protuberance may undergo elongation sufficient to alter the characteristic shape of the axile ovule (fig. 2). Occasionally in *I. crenata*, the axile and parietal ovules show equal development especially in the early phases of growth (fig. 9). Marked growth during any stage of maturation, however, confined to one of the two ovules leads to the distortion or obliteration of the other.

These events indicate a definite tendency for a biovulate condition in the loculus of some *Euilex* species. The only extant species of *Ilex* which typically produces two ovules in each loculus is *I. teratopis* Loes. of the monotypic subgenus *Yrbonia*. According to Loesner (1942), *I. teratopis* which is narrowly restricted to the central Andean region of South America closely resembles neighboring *Euilex* species except for the distinct biovulate condition. Consideration of these several facts has led to the conclusion that the biovulate condition of infrequent occurrence in modern forms of *Euilex* was once prevalent among ancient species of this subgenus. *I. teratopis*, therefore, is probably a relict species of this ancient *Euilex* group.

Evidence reported here is in agreement with the widely held view that the axile position of the ovule is the typical condition in *Euilex*. Thus, the transition from a biovulate to a uniovulate condi-

tion in this subgenus has resulted from suppression of the parietal second ovule.

There are several structural features associated with the ovules of *Ilex* which may indeed reflect the typical conditions in the remote ancestor of the genus. Sectioned ovules from several species frequently disclose two distinct vascular strands in the broad base of the funiculus (fig. 7, 16). In *I. cornuta*, the funicular base is considerably thicker at its upper and lower extremities than at its center and appears, therefore, to be formed by the fusion of two structures (fig. 13). These two features may be significantly related to a third phenomenon observed in both *I. crenata* and *I. vomitoria*. Preparations from these two species show the formation of a second ovule from the funicular protuberance of the established first ovule. This second ovule shows abnormal features in its development, and it frequently enlarges sufficiently to compress and distort the first ovule (figs. 14, 15).

These structural and developmental features suggest that the ovule in modern species of *Ilex* actually represents an incorporation of two ovules. One member of this incorporate structure completes full, normal development while the other member, partially suppressed, forms the funicular protuberance. However, as indicated previously (figs. 14, 15), the suppressed ovule, i.e. the funicular protuberance, retains the potentiality for normal development.

This latter hypothesis when considered in terms of the biovulate tendency discussed previously leads to the following conclusion. In remote times, the loculus contained four ovules, two from the axile and two from the parietal surface. The distance between the members of each pair was subsequently shortened leading ultimately to the close approximation of their once separate points of origin. Thus, in the modern ovulary a single rudimentary ovule on either locular surface represents two ovular primordia in such close association and with one member markedly suppressed that visible evidence of the dual origin is completely erased. Nevertheless, subsequent ontogenetic development reveals that both of the ovular primordia maintain a definite tendency toward maturation, and indeed both contribute to the final configuration of the *Ilex* ovule.

Rapid growth confined to only one of the two fused members retards growth in the other and forms the functional nucellus and the integument. The funicular protuberance is considered the principal portion of the retarded member and is equivalent to the nucellus and integument of the functional member. This view is certainly suggested by the occasional formation of a second ovule from the funicular protuberance. The presence of two vascular strands in the funiculus provides strong evidence for the proposed dual origin of the ovule.

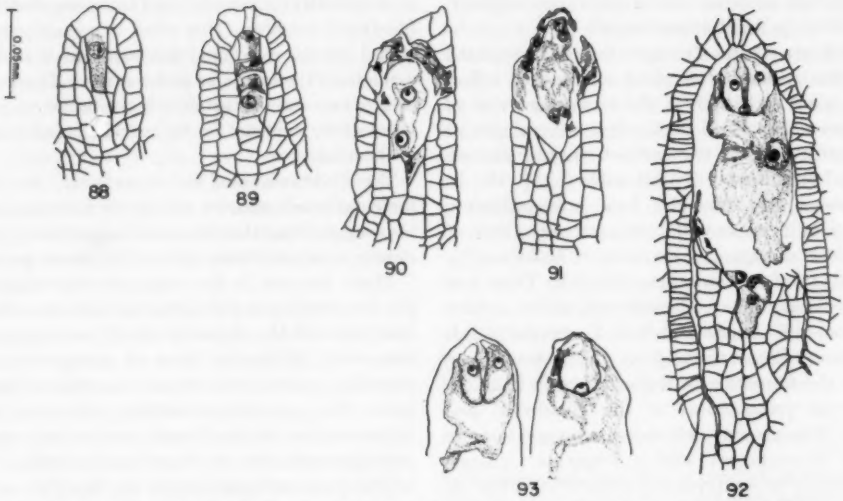
The conditions noted in *I. cornuta, viz.*, that the funicular base is massive and shows uneven thickness suggest that the two ovule primordia are less closely associated here than in the other species.

Many features in the proposed dual origin of the *Ilex* ovule are still somewhat obscure. Clear elucidation of the situation will require an extensive study of species from all groups focused especially on the early phases of ovule development. The application of clearing techniques and in general the study of whole, unsectioned ovule and placental material should prove useful.

The proposed dual origin for the *Ilex* ovule contains some broad implications pertinent to ovule origin in Angiosperms. Generally held is the concept that the reduction in the multiovulate condition to establish finally the uniovulate carpel represents the absolute loss of ovules. In view of the observations here, this numerical reduction may have resulted from ovule union in addition to partial suppression, and, thus, the Angiosperm ovule may derive its modern expression from two or more fused ovule primordia. There is, however, the distinct possibility that the incorporation of ovules is peculiar only to specific groups of plants and represents in these forms merely a transitional phase toward complete suppression.

Several points mentioned here assume importance in the light of Loesner's interpretation of the funicular protuberance which, by his reference, is the funicular "cap structure". In his 1919 treatment, Loesner regarded the funicular protuberance as a reduced aril of the type found among the drupaceous Celastraceae in the subfamily Cassinoideae. As previously mentioned, the funicular protuberance in *Ilex* grows from the entire inner surface of the funiculus while, according to Loesner, the aril of the Cassinoideae originates at the hilum. He considers this difference in origin too insignificant to disallow homology between the two structures. With the retention of his view, the drupaceous Cassinoideae become the closest relatives of Aquifoliaceae, and in Loesner's account derivation of the latter from the former is certainly inferred.

Consideration of the funicular protuberance as a reduced aril appears contrary to the proposal



FIGS. 88–93. *Ilex decidua* Walt. Fig. 88. Hypodermal megaspore mother cell. ×267. Fig. 89. Four megaspores in linear sequence, hypodermal. ×267. Fig. 90. Two-nucleate phase; disintegration in micropylar nucellus. ×267. Fig. 91. Four-nucleate phase; advanced disintegration in micropylar nucellus. ×267. Fig. 92. Mature megagametophyte; synergids enlarged, obscure egg (cf. fig. 93). ×267. Fig. 93. Micropylar portion of mature megagametophyte (cf. fig. 92) shown in two consecutive sections; synergids (left) enlarged and vacuolate. ×267.

stated here, namely, that it is a suppressed, nonfunctional ovule. The difference in the two viewpoints, however, would be removed if, indeed, the cassinoidean aril were also of ovular origin. It is not inconceivable that ovule union occurred in the ancestral Cassinoideae, and that the retarded member of this fusion produced the funicular aril. In this regard, the aril and the funicular protuberance may actually be homologous—both nonfunctional ovules. The cassinoidean aril, according to Loesner, is a more highly developed structure than is the *Ilex* funicular protuberance. If it is ovular in origin, the aril must be regarded as suppressed in function only. Developmentally, however, it is not suppressed but follows instead a new, secondary pattern of growth and at maturity envelops the seed. Perhaps the radial elongation of the protuberance cells in *I. cornuta* (figs. 11, 12) represents the initial phase in a similar growth pattern.

The wood anatomical data collected by Pennington (1953) and Boole (1955) support the view that Aquifoliaceae and Celastraceae are related, and such relationship is also strongly indicated by several features in megagametogenesis mentioned in previous discussion. The striking points of similarity may be summarized as follows: the formation of sporogenous tissue; the structure and growth pattern of the nucellus; the arrangement and behavior of the megaspores; megagametogenesis, especially the behavior of the synergids, polar nuclei, and antipodal cells. The number of integuments (unitegmic ovules in *Ilex*, a bitegmic condition in Celastraceae) constitutes a major point of dissimilarity between the two groups.

In earlier discussion, a derived rather than a generalized status was proposed for Celastraceae on the basis of embryological data. Chief among these features is the crassinucellate condition which though mildly expressed in both families is more prominent in Aquifoliaceae.

In addition, the anatomical data collected by Pennington (1953) and Boole (1955) are directly contrary to Loesner's proposed derivation of Aquifoliaceae from Celastraceae. With regard to vessel characteristics, length, diameter, wall thickness, and end wall angle, the generalized members of Celastraceae especially those in the subfamily Cassinoideae are considerably more derived than the generalized members of Aquifoliaceae.

Berkeley (1953) reports that the disc of Celastraceae has originated from the adnation of suppressed floral parts. Such adnation constitutes

another mark of advancement in Celastraceae which is entirely absent in Aquifoliaceae.

Furthermore, the restricted point of origin of the cassinoidean aril in addition to its extensive development indicates that this structure is more advanced than its supposed homologue, the funicular protuberance of *Ilex*.

It is, therefore, suggested that the preponderance of evidence points to a more generalized position for Aquifoliaceae, but this suggestion is not intended to imply direct derivation of Celastraceae from Aquifoliaceae. On the contrary, both groups are here considered derivatives of a common, remote ancestor. This view is necessitated by the fact that the bitegmic condition of Celastraceae is accepted to be more generalized than the unitegmic condition characteristic of Aquifoliaceae.

SUMMARY

The series of events which compose the typical pattern in the development of the ovule and megagametophyte of *Ilex* is summarized in the following diagnosis:

Placentation axile, rarely parietal; ovule unitegmic, anatropous; funiculus broad with prominent protuberance on its entire inner surface. Sporogenous tissue of one to three cells formed from a single primary archesporial cell after initial curvature of the ovule; megaspore mother cell derived from a sporogenous cell or by direct enlargement of the primary archesporium. Four megaspores produced in linear sequence with the chalazal spore functional; nucellus mildly crassinucellate with maximal growth during megasporogenesis. Megagametogenesis after the *Polygonum* type; disintegration of the micropylar nucellus effected at the eight-nucleate phase; antipodal cells early distorted, frequently persist through early endosperm development, rarely enlarged and vacuolate; polar nuclei fuse early; synergids persistent, seldom enlarged and vacuolate.

The major developmental variations are associated with the formation of sporogenous tissue, growth of the nucellus, and structure of the ovules. Observations are too infrequent to allow categorical evaluation of the variable features for their taxonomic significance.

Features having special significance are the occasional appearance of biovulate loculi and the distinctive funicular protuberance.

The funicular protuberance considered by Loesner to be a reduced aril appears in the light of evidence here adduced to be a suppressed, nonfunctional ovule.

The striking points of similarity between Aquifoliaceae and Celastraceae are: the formation of sporogenous tissue; the structure and growth pattern of the nucellus; the arrangement and behavior of the megaspores; and the development of the megagametophyte, especially the behavior of the synergids, polar nuclei, and antipodal cells. The number of integuments constitutes a major point of dissimilarity between the two groups.

Development observed here with other morphological features indicates a close relationship between Aquifoliaceae and Celastraceae with the former undoubtedly more primitive than the latter, contrary to the view of Loesner.

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IPOMOEA TRICHOCARPA ELL. AND *IPOMOEA TRIFIDA* G. DON

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Two species of *Ipomoea* (*trichocarpa* and *trifida*) although widely separated in Small's Manual of the Southeastern Flora are very closely related, in fact so closely that I can see no reason for maintaining two specific entities. This matter was drawn to attention when on trying to identify a particular *Ipomoea*, collected for the Flora of the Carolinas project, it was noted that the plants would not key nor fit the description satisfactorily. The plants had large perennial roots and sepals that were heavily pubescent on the back as well as being ciliate. The separating point in the Small (1933) key is, "Perennial: calyx glabrous", for *I. trifida* as opposed to, "Annual: calyx pubescent, at least the sepals ciliate" for *I. trichocarpa*. House (1908) in his monograph on the genus Ipomoea used the same characters to separate the species, but adding "corolla 3 cm. long or longer" for *I. trichocarpa*, as opposed to "corolla 2–3 cm. long" for *I. trifida*, However, *I. trifida* has a var. *torreyana* Gray that has corollas 4 cm. long. Therefore the length of the corolla is of no significance.

In the original description of *I. trichocarpa* by Elliott, it is definitely stated that the root is annual and the calyx is ciliate and slightly hairy. The description for *I. trifida* by G. Don states that the sepals are glabrous and the plant is a perennial. It is very likely that either of these two species could flower when quite young and hence have been interpreted as an annual or it is just as likely that at times given plants could actually be annuals.

In the hope of seeing some differences on herbarium sheets, material was borrowed from the Smithsonian Institution, Washington, D. C. With all of this material at hand however I am still unable to find any constant, reliable character on which to maintain two species. The name on the basis of priority should therefore be *Ipomoea trichocarpa* Ell.

The material from the Carolinas is represented by specimens that show much variation in indument of stems, leaves, sepals, and capsules, as well as lobing of leaves. One specimen in which the entire plant is totally glabrous seems hardly worthy of recognition, although two others seem worthy of formal status.

Two New Forms of *Ipomoea trichocarpa* Ell.

Ipomoea trichocarpa forma *albiflora* forma nov. Corolla white, foliage and stems without anthocyanin. Corollis albidis.

Holotype: Colleton Co., South Carolina: low pine-oak woods border, 1.1 miles east of junction of County Route 90 and 40 on County Route 90, September 5, 1956, *Ahles 17956*.

Ipomoea trichocarpa forma *pubescens* forma nov. Leaves on both surfaces, stems, petioles, peduncles, calyces and capsules all densely pustulate-pilose. Tota planta dense pustulata-pilosa.

Holotype: Calhoun Co., South Carolina: thicket, Halfway Swamp on South Carolina Route 6 (south of Lone Star), September 27, 1957, *Ahles 35245*.

Specimens are deposited in the Herbarium of the University of North Carolina.

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ARISTOLOCHIA SERPENTARIA VAR. *NASHII* AS A NEW NAME FOR *ARISTOLOCHIA SERPENTARIA* VAR. *HASTATA*

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Aristolochia serpentaria var. *hastata* (Nutt.) Duchartre is an illegitimate name as it was based on *Aristolochia hastata* Nutt. which is preceded by a Mexican species *A. hastata* HBK. Since there is no earlier name in the varietal category for this plant, a new name must be erected. The name *Aristolochia nashii* Kearney is here taken as the basonym for the new combination *Aristolochia serpentaria* var. *nashii* (Kearney) comb. nov. According to Duchartre in De Candolle, Prodromus Systematis Naturalis, *Endodeca polyrrhizos* Klotzsch is also a synonym of this taxon. Although this name is the next earliest epithet after *Aristolochia hastata* it is not here used for the basonym for a new combination since I am uncertain of its application, not having seen the publication. It is therefore listed as a questionable synonym.

Synonymy of *A. hastata* var. *nashii* (Kearney) Ahles

Aristolochia hastata Nutt. 1818. Gen. N. A. Pl. 2: 200—Non HBK.

? *Aristolochia polyrrhizos* Spreng. 1827. Syst. 4, pars 2: 310.

Endodeca hastata (Nutt.) Raf. 1836. Fl. Tellur. 12: 99.

? *Endodeca polyrrhizos* (Spreng.) Klotzsch, 1859 in Monatsbericht Acad. Berl. 601.

Aristolochia serpentaria var. *hastata* (Nutt.) Duchartre 1864 in De Candolle, Prod. Syst. 15: pars 1: 434.

Aristolochia sagittata Muhl., Duchartre 1864 in De Candolle. loc. cit.—name used in synonymy. Apparently only a label name.

Aristolochia nashii Kearney 1894 in Bull. Torr. Club 21: 485.

Cleistogamy in *Aristolochia*

It is of interest to note that what appears to be cleistogamy is rather common in *A. serpentaria* var. *nashii*, although there seems to be no note to this effect published in our present literature. Cleistogamy was noticed only in the variety, but further observations may reveal that it is to be found throughout the species and perhaps closely related species. The cleistogamous flowers are quite small, less than 5 mm. long and about as wide, tapering to a slightly pointed apex.

A NATURAL STAND OF *RHODENDRON* X *WELLESLEYANUM* WATERER EX REHDER IN THE SOUTHERN APPALACHIANS

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Rhododendron catawbiense Michx. has been widely used by horticulturists in the formation of new hybrid varieties and some use has been made of *R. maximum* L. These two species are usually separated in nature by differences in flowering dates and altitudinal distribution, but slight overlapping does occur. No report of a natural occurrence of a hybrid between these two species could be found by the writer in a survey of the manuals of the region or in horticultural publications.

The hybrid plant resulting from a forced cross of *R. catawbiense* and *R. maximum* was given the name *R. wellesleyanum* by Waterer in 1880 and was published as a nomen subnudum in Rehder's *Manual of Cultivated Trees and Shrubs* in 1927.

On June 27, 1957, the writer observed a stand of *R. X wellesleyanum* at an elevation of 5600 feet on Bear Ridge in the Black Mountains of North Carolina. This stand is composed of 12 shrubs of a variable number of stems per plant. The individual plants are about the same height, indicating the probability that this entire stand is the progeny of a single flower which was cross pollinated. The stand is confined to an irregular area with a maximum radius of about 40 feet. There are no morphological variations to indicate introgression. Plants of *R. catawbiense* and *R. maximum* are intermixed with those of the hybrid. The former is near its lower altitudinal limit, the latter near its upper altitudinal limit. This stand is in an area which was burned over about 40 years ago, and the canopy is now dominated by fire cherry (*Prunus pensylvanica* L.f.) and yellow birch (*Betula lutea* Michx. f.). When first observed the stand was at the peak of its blooming season. Only an occasional flower of *R. catawbiense* remained and only a few flowers of *R. maximum* had opened. The hybrid plants are intermediate in leaf shape and undersurface vestiture, flower size and color, and viscidness of the pedicel.

Specimens from the hybrid stand, as well as from *R. catawbiense* and *R. maximum* from the immediate area, are on file in the herbarium of the University of North Carolina. A plant of the hybrid was transplanted to Burke County, N. C. near Morganton, at an elevation of about 1100 feet. The plant has shown considerable growth during the first two years after transplanting.

NOTES ON THE VASCULAR FLORA ALONG THE LOWER DEEP RIVER OF NORTH CAROLINA[1]

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In collections made along the lower Deep River, several plants with montane and northern affinities, and several typical of the Coastal Plain, were found. The most striking of the montane species were *Camptosorus rhizophyllus* (L.) Link, *Pinus strobus* L., *Corallorhiza wisteriana* Conrad, *Isopyrum biternatum* (Raf.) T. & G., *Dicentra cucullaria* (L.) Bernh., *Cubelium concolor* (Forst.) Raf., *Hydrophyllum virginianum* L., *Nemophila microcalyx* (Nutt.) Fisch. & Mey., and *Campanula divaricata* Michx.; the outstanding Coastal Plain elements were *Zizaniopsis miliacea* Döll & Aschers., *Quercus michauxii* Nutt., *Vaccinium crassifolium* Andr., *Seymeria cassioides* (Walt.) Blake, and *Dicliptera brachiata* (Pursh) Spreng. Also discovered here was *Ptilimnium fluviatile* (Rose) Mathias, a usually montane species of disjunct distribution, known from one other locality in North Carolina.

The Deep River flows entirely within the Piedmont Province, but near Glendon, it flows out of the Piedmont Plateau into the Deep River Triassic Basin. It enters a wedge-shaped extension of the latter about 1.4 miles upstream from Woodward Bridge (U. S. 15-501), and again enters the Piedmont Plateau at the termination of the wedge about one-quarter of a mile below the bridge. Except for a slight swiftness at this point, where the river crosses a fault in the bedrock, the water flows quietly to a point about .6 of a mile below the junction of the Deep and Rocky Rivers; but from the latter place, the river is characterized by exposed rock and rapids to as far as U. S. 1 bridge just below Lockville Dam, excepting about one-half mile of inundated river bed above the dam. The disappearance of the rapids and the presence of an escarpment mark the place where the river, for the second time, issues from the Piedmont Plateau into the Triassic Basin. In this basin the water flows on more calmly to its confluence with that of the Haw River to form the Cape Fear. The area studied is a narrow strip in Chatham and Lee Counties consisting of the alluvium and adjacent slopes along the Deep River and its tributaries, from the union of the Deep and Rocky Rivers to the union of the Deep and Haw Rivers about 3.6 miles downstream.

The Piedmont Plateau consists of the remains of peneplained mountains, the rocks of which are represented as of pre-Cambrian or Lower Paleozoic age on the geologic map of North Carolina. The soils of the uplands adjacent to the Deep River are sedentary, consisting chiefly of clay and silt loams. In several places, diabase dikes occur, and the red clay loam derived from them is the richest soil in the area.

The slopes in the Piedmont Plateau at the junction of the Deep and Rocky Rivers represent the easternmost station in North Carolina for *Pinus strobus* L. This pine has been almost obliterated in the locality by cutting. Tree-size specimens are scarce now, but saplings and seedlings still persist in small numbers. Higher ridges and steeper bluffs occur along the Rocky River than occur along the Deep River, and the remaining white pine persists on the ridges and bluffs for a greater distance along the Rocky River than it does along the Deep.

On damp, north-facing slopes and banks in the above vicinity in Lee County, *Camptosorus rhizophyllus* (L.) Link, *Adiantum pedatum* L., *Chamaelirium luteum* (L.) Gray, *Amianthium muscaetoxicum* (Walt.) Gray, *Hydrangea arborescens* L., *Dirca palustris* L., *Campanula americana* L., and *C. divaricata* Michx. occur. One specimen of *Lygodium japonicum* (Thunb.) Sw. and one of *Ilex montana* T. & G. were collected here. *Scleria oligantha* Michx., *Corallorhiza wisteriana* Conrad, *Quercus shumardii* Buckl., *Asarum canadense* L. var. *reflexum* (Bickn.) Robins., *Tilia heterophylla* Vent., *Panax quinquefolius* L., *Chimaphila umbellata* (L.) Bart. var. *cisatlantica* Blake, and *Symplocus tinctoria* (L.) L'Her. were found on the rich, wooded slopes. On the alluvium in this part of the area, *Botrychium virginianum* (L.) Sw., *Ophioglossum vulgatum* L. var. *pycnostichum*

[1] From a thesis submitted to the faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Botany. The study was performed under the guidance of Dr. A. E. Radford.

Fern., *Festuca paradoxa* Desv., *Myosurus minimus* L., *Anemone lancifolia* Pursh, *Sedum ternatum* Michx., and *Cubelium concolor* (Forst.) Raf. are occasional in occurrence. *Podostemum ceratophyllum* Michx. forms mats on the submerged rocks in the rapids.

The rich clay loam along diabase dikes supports vegetation of a somewhat different character from that of most of the other soils. Along these dikes, *Juniperus virginiana* L., *Juglans nigra* L., *Carya carolinae-septentrionalis* (Ashe) Engl. & Graebn., *Quercus rubra* L., *Aristolochia serpentaria* L., *Elaeagnus umbellata* Thunb., and *Sanicula gregaria* Bickn. are decidedly more common than elsewhere in the area. *Gentiana villosa* L., *Monarda fistulosa* L. var. *mollis* (L.) Benth., and *Cirsium pumilum* (Nutt.) Spreng. were found only on this soil.

A site behind Lockville Dam is affected by its position on the boundary between the Piedmont Plateau and the Triassic Basin, and by the presence of the dam. This concrete dam is about ten feet high, and is founded on the exposed rock. It harnesses water for the operation of a hydroelectric plant about five hundred yards downstream, to which the water is shunted by means of a canal. During the daytime, when the electric plant is in full operation, no water pours over the dam except when the river is high. Ordinarily, water flows in the river bed at night when operation of the plant is reduced. As a result of ths nightly flooding, the sand and gravel in much of the bed is wet most of the time. Here *Salix caroliniana* Michx., *Scirpus americanus* Pers., *Ptilimnium fluviatile* (Rose) Mathias, and *Dracocephalum virginianum* L. are plentiful. The willow appears to be a staminate clone. One specimen of *Zizaniopsis miliacea* (Michx.) Döll & Aschers. was collected.

It is significant that *Ptilimnium fluviatile* was found to be rare in wet gravel deposits among the rapids farther upstream. Limited searching in apparently similar situations in the Rocky and Haw Rivers failed to reveal its presence in those places. This same species was collected in the Tar River in wet sand (Ahles, *et al.*, 1958). It is significant also that the locality in the Tar River is in the Piedmont Plateau about .1 of a mile east of the boundary of the Piedmont Plateau and the Triassic Basin.

The Triassic Basin lies in the edge of the Piedmont Plateau, and geographically is a part of the Piedmont Province, though it has some geological and vegetational similarities to the Coastal Plain which borders it to the southeast. The topography of the basin within the study area is flat to slightly rolling. The soils are chiefly alluvial, consisting of sandy and silt loams, the remains of post-Triassic terrace deposits (Reinemund, 1955). These soils are more poorly aerated and more poorly drained, both externally and internally, than those of the Piedmont Plateau, and consequently present different types of habitats. For example, the portion of the area in the Piedmont Plateau supports typical climax and postclimax forests for the Piedmont (Oosting, 1942), while that in the Triassic Basin supports typical preclimax forest (*loc. cit.*).

The two most extensive terraces on the Lee County side of the river south of Moncure, from Lonnie Wombles Creek northeastward to the Seaboard Railroad, and bounded on the southwest by a county road, were studied. The lower of these consists of a compact silt loam that underlies a fine sandy loam of the upper terrace, but the former is exposed in large patches where the latter has been removed by erosion. The silt loam is more impervious to water, and frequently serves as a moisture reservoir or drainage way between remnants of the upper terrace, and, as such, exhibits the character of a marsh, often with an abundance of *Sphagnum*. The outlying patches of the lower terrace and the sandy loam of the upper terrace tend to be dry. The slightest depressions in the sandy loam, however, such as may be caused by the compacting of the soil by heavy vehicles, or by the grading of road rights of way, present an aspect similar to that of the wetter silt loam in that moisture accumulates sufficiently to support several marsh species.

Some plants occurring in wet or damp situations in the terrace soils are *Uniola laxa* (L.) BSP., *Cyperus virens* Michx., *Rhynchospora corniculata* (Lam.) Gray, *Juncus repens* Michx., *Spiranthes praecox* (Walt.) S. Wats., *Magnolia virginiana* L., *Callitriche heterophylla* Pursh, *Vaccinium crassifolium* Andr., *Lycopus virginicus* L., and *Lobelia elongata* Small. On drier sites, *Comandra umbellata* (L.) Nutt., *Pyrus arbutifolia* (L.) L. f., *Vernonia acaulis* (Walt.) Gleason, *Eupatorium semiserratum* DC., and *Aster spectabilis* Ait. frequently occur, the latter two only along road cuts, while *Crotalaria purshii* DC., *Seymeria cassioides* (Walt.) Blake, *Gerardia purpurea* L., *Solidago austrina* Small, *S. nemoralis* Ait., *Aster gracilis* Nutt., and *Cirsium virginianum* (L.) Michx. are occasional or rare in occurrence.

Several interesting species occur in the low-

lands along the river in the Triassic Basin. *Nemophila microcalyx* (Nutt.) Fisch. & Mey. is frequent on northerly exposed floodplains, as is *Dicliptera brachiata* (Pursh) Spreng. on the banks. *D. brachiata* also occurs along the Deep River in the portion of the Triassic Basin to the west of the study area, but not in the intervening part of the Piedmont Plateau. *Lamium purpureum* L. is plentiful at a site just below the Seaboard Railroad bridge in Lee County. *Aneilema keisak* Hassk., *Polygonatum canaliculatum* (Muhl.) Pursh, *Smilax walteri* Pursh, *Phyllanthus caroliniensis* Walt., and *Hydrophyllum virginianum* L. are occasional to rare on floodplains and damp banks, while *Carex joori* Bailey, *Juncus platyphyllus* (Wieg.) Fern., *Physocarpus opulifolius* (L.) Maxim., *Osmorhiza longistylis* (Torr.) DC. var. *villicaulis* Fern., and *Helenium autumnale* L. var. *parviflorum* (Nutt.) Fern. occur with similar frequence in the river bottoms.

On the floodplain of the Haw River in the vertex of the angle formed by the confluence of the Deep and Haw Rivers, six tree-size specimens of *Gymnocladus dioica* (L.) K. Koch were found. One specimen, about fourteen inches in d.b.h., was broken off about twelve feet from the ground, and had suckers growing from the trunk. A second specimen was about nine inches in d.b.h., and had numerous seedling-size specimens distributed about it. The remaining specimens were five to six inches in diameter. No evidence of fruiting was seen in the three seasons that these trees were observed.

Several notable species are found in the area in both the Piedmont Plateau and the Triassic Basin. On the rich slopes along the river occur *Quercus michauxii* Nutt., *Laportea canadensis* (L.) Wedd., *Isopyrum biternatum* (Raf.) T. & G., *Dicentra cucullaria* (L.) Bernh., and *Acer saccharum* Marsh., the first mentioned species also occurring on the rich uplands, well removed from the river, in the Piedmont Plateau. *Lysimachia nummularia* L. is common in low damp woods, and *Hypericum lloydii* (Svenson) Adams is abundant in the rocky and more sterile soils.

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A FLORISTIC STUDY OF A SANDHILL AREA ON THE NORTH CAROLINA COASTAL PLAIN

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Sandhills occur on the Southeastern coastal plain in those areas where loose coarse sands coincide with a rolling topography which may vary from low ridges to comparatively high hills. The formation reaches its highest expression in the Upper Pleistocene terraces of Moore, Cumberland, Hoke, and Harnette counties where the "sandhills climate" has been recently much exploited commercially. Sandhills may also be found in more limited areas on the lower terraces; one such area was noted by A. E. Radford during the summer of 1957 while collecting for the *Flora of the Carolinas*, and, as the only previous botanical work in the area was a notation by Fox and Godfrey (1951) of Wayne County as a third station for *Chrysopsis pinifolia*, the area was selected for a more intensive floristic study.

Sandhills were found in southern Wayne and Lenoir counties, for the most part south of the Neuse, extending southward into the northern part of Duplin County. The western limit of the sandhills in Wayne County corresponds roughly with a line drawn between Dudley and Williams' Pond; the eastward limit is in the vicinity of Moss Hill School in Lenoir County.

Geology, Climate, and Soils

Wells and Shunk (1931) have stated that the majority of the sandhill deposits represent the work of water along estuaries, shores, and river beds. This is apparently the case in the Wayne-Lenoir-Duplin County sands where, overlying the Upper Cretaceous Black Creek formation of interbedded dark clays and organic sands, there is a wide superficial deposit of coarse sand which may vary from three to one hundred feet in thickness. These sands are of the Norfolk series. To a depth of six to eight inches they consist of a light grey to nearly white medium sand beneath which is a pale yellow to yellow loose porous sand. Less than an inch beneath the white inorganic surface sand is a thin layer of blackish organic matter which Wells and Shunk (1931) have shown to be in large part derived from the root systems of the surrounding clumps of wiregrass.

The soils of the bays or pocosins are quite varied and have not been extensively studied.

The rolling topography, essential for the development of typical sandhills communities, results from the interaction of the Surrey Scarp, separating the lower Pamlico Terrace from the higher sands of the Talbot Formation, with the Neuse River.

Climatic records from the nearest station, Goldsboro in Wayne County, show a mild and uniform climate with a mean annual temperature of 61.1 degrees Fahrenheit with a recorded winter low of 2 degrees and a summer high of 108 degrees. The average precipitation is 52.87 inches and the growing season lasts about 240 days.

a. The Xeric Ridge Community

On the more xeric ridges, the dominant woody species is the longleaf pine (*Pinus australis*). In the area directly south of the Neuse, this pine has not been cut-over completely, and open stands which appear to have been regularly logged and rather well managed have been maintained over a large area.

Numerous sprouts of the turkey oak (*Quercus laevis*) were present. While in surrounding dry sandy flatland areas there seemed to be a direct successful relationship between the pine and the more xeric of the oaks, this was not apparent in the sandhills. There were no mature turkey oaks nor were there any seedlings; most of the plants were shrub-sized specimens with thickened lower stems and roots which gave evidence of fire damage. Apparently the longleaf pines are of a fire-maintained disclimax (pyroclimax), and the appearance of the turkey oaks as true dominants in the canopy would occur only in the absence of recurring fires.

Theoretically, after centuries of no fire whatever, the sandhills would move in the direction of a deciduous forest composed largely of the white-heart hickory (*Carya tomentosa*) which, in the opinion of Wells and Shunk (1931), would be truly climatic for the area. It would seem, however, that fire was a common factor, even when

the sandhills were undisturbed by white men, and apparently the extreme xericity is self-maintaining through this prevention of the accumulation of the organic matter which would be essential to the further and ultimate establishment of hardwoods.

Wiregrass (*Aristida stricta*), along with several species of *Andropogon*, including *A. ternarius*, *A. virginicus*, *A. scoparius*, and *A. elliottii*, is the dominant herb. These grasses are always found in clumps six inches or so in diameter, and the clumps are well separated by patches of bare sand. While the lichen *Cladonia sylvatica* appears to pioneer on these bare sand areas, along with such xerically adapted herbs as *Arenaria caroliniana*, *Asclepias amplexicaulis*, *Stipulicida setacea*, and the grass stage of the longleaf pine, below the sand surface these areas are well interlaced with the roots of the wiregrass, and it is highly unlikely that a true succession as such follows the establishment of these "pioneers."

A small shrub which is able to compete successfully with the wiregrass is the dwarf huckleberry, *Gaylussacia dumosa*, which may form large colonies over considerable areas.

In early summer and late fall, *Chrysopsis pinifolia*, a plant quite rare in other sandhills areas, shares aspect dominance with the clumped grasses over many acres of the Wayne County sandhills where, in the frequently burned and harvested longleaf pine woods, conditions are apparently favorable to its existence.

An interesting species collected by A. E. Radford in 1957 from the Wayne County sands is *Carex tenax*, the nearest station for this plant of the xeric ridges being some hundred miles distant across the South Carolina border. Radford also collected the live oak (*Quercus virginiana*) from a ridge in Wayne County. This collection represents a northward and inland extension of the range of this predominantly southern and coastal plant.

Fifty-three species were found restricted to the xeric ridges. Prominent among these were *Amorpha herbacea*, *Aristida lanosa*, *Asclepias amplexicaulis*, *A. humistrata*, *Ascyrum hypericoides*, *Aster concolor*, *A. dumosus*, *A. linariifolius*, *Aureolaria pectinata*, *Baptisia villosa*, *Bulbostylis barbata*, *Carphephorus tomentosus*, *Cenchrus pauciflorus*, *Chrysopsis gossypina*, *Cirsium repandum*, *Cnidoscolus stimulosus*, *Crotalaria angulata*, *Digitaria sanguinalis*, *Erigeron ramosus*, *Euphorbia maculata*, *Helianthemum canadense*, *Hypericum galioides*, *H. gentianoides*, *Indigofera caroliniana*, *Lespedeza hirta*, *L. stuevei*, *Linaria floridana*, *Petalostemum pinnatum*, *Rhynchosia simplicifolia*, *Tephrosia spicata*, *Yucca filamentosa*, and amazingly, on some of the most xeric sands and roadside areas, the watermelon, *Citrullus vulgaris*.

b. The Bay or Pocosin Community

At the foot of the ridges where the underlying clay strata approach the surface of the soil and the water table is often, especially in times of high rainfall, above ground, the pocosin or bay communities are found. In the smaller pocosins, the dominant shrubs are the elliptic-leaved evergreen shrubs, mostly hollies or members of the Ericaceae. Among these are *Clethra alnifolia*, *Cyrilla racemiflora*, *Ilex coriacea*, *I. glabra*, *I. verticillata*, *Leucothoe axillaris*, *L. racemosa*, *Lyonia mariana*, *Myrica cerifera*, *Viburnum nudum*, and *Zenobia pulverulenta* forma *nuda*. Of these, *Ilex glabra* is most common and widespread.

In the larger pocosins, a bay forest develops with *Liquidambar styraciflua*, *Magnolia virginiana*, and *Nyssa sylvatica* dominant in the canopy. *Acer rubrum*, *Liriodendron tulipifera*, and large specimens of *Ilex opaca* are common though never dominant, and the pond pine (*Pinus serotina*), though essentially a plant of the ecotone between pocosin and ridge, may extend well into some bays.

Beneath the bay forest, the dominant herb is the cinnamon fern, *Osmunda cinnamomea*; where a pocosin has burned over, the cane, *Arundinaria tecta*, occurs in large stands. Typical pocosin herbs include *Carex folliculata*, *Cuscuta compacta*, *Juncus biflorus*, *J. effusus*, *Polygonum hydropiperoides*, *Sarracenia flava*, and *S. purpurea*. *Juncus longii*, reported in Batson's monograph of the rushes of North Carolina (1952) as infrequent in the state, was collected from a pocosin in Wayne County, as was another species rare to sandhills, the fly poison, *Amianthium muscaetoxicum*. *Smilax laurifolia* is the typical pocosin catbrier which literally ties the community together; *Smilax rotundifolia* is also present.

c. The Ecotone Community

The transitional areas between the ridges and the pocosins are, like most ecotonal areas, quite rich in species, possessing elements unique to themselves as well as members of both adjoining communities. It is difficult to delineate a definite zonation of species along a moisture gradient

since xeric species may, during periods of adverse moisture conditions, penetrate well into the pocosin and remain there, living if not flourishing, after the water table has risen, while typically pocosin species, with perhaps less success, may establish themselves well out onto previously xeric sands during periods of high rainfall. Hence the cinnamon fern may be found at the edges of the pocosin stream where it is the dominant herb with fronds six to eight feet in height and also at the xeric fringes of the ecotone where it is represented by scattered specimens with frond lengths of less than two feet.

The dominant woody species of the more xeric portions of the ecotone may well be derived from the more xeric elements of the Piedmont flora. They include *Carya tomentosa*, *Quercus incana*, *Q. marilandica*, and *Q. stellata* var. *margaretta*. *Sassafras albidum*, *Crataegus uniflora*, *Prunus angustifolia*, *Cornus florida*, and such shrubs as *Callicarpa americana*, *Ilex opaca*, *Myrica cerifera*, *Rhus copallina*, *Symplocos tinctoria*, and *Vitis rotundifolia* are also present.

The pond pine is the dominant tree in the more mesic portions of the ecotone. In addition to many of the typically pocosin species, a large number of shrubs are found including *Amelanchier obovalis*, *Rhododendron atlantica*, *Itea virginica*, *Kalmia angustifolia*, *Persea borbonia*, *Pyrus arbitufolia*, *Rubus enslenii*, *Vaccinium corymbosum*, and *V. stamineum*. In addition to the pond pine, there are several herbaceous species typical of savannah regions; these are *Drosera brevifolia*, *Lachnocaulon anceps*, and *Lycopodium alopecuroides*.

The total number of ecotonal species is lengthy; a few which occur with high fidelity are *Aster paludosus*, *Gentiana autumnalis*, *Juncus scirpoides*, *Liatris graminifolia*, *Mitchella repens*, *Polygala lutea*, *Pyxidanthera barbulata*, *Rhexia ciliosa*, *R. mariana*, *Rhynchospora cephalantha*, *R. inexpansa*, *Trilisa odoratissima*, *Xyris flexuosa*, and *X. platylepsis*.

d. Disturbed Communities

Three types of disturbed communities (other than that of fire disturbance which has been previously discussed) were observed—cut-over pine woods, old fields, and roadsides.

Other than the occurrence of *Chrysopsis pinifolia* in large tracts, the floristics of the cut-over pine woods were not markedly different from those of the xeric ridges in general.

Old fields are not particularly common in the area. Typical old field species are *Happlopappus divaricatus*, *Digitaria sanguinalis*, the sandhills crabgrass, and *Bulbostylis barbata*. The latter, a small tufted sedge, is interesting in that, previous to the collections made in preparation of the *Flora of the Carolinas*, it was known only from Florida and Georgia within the United States. In the Wayne-Lenoir-Duplin County sands, it occupies the same sites as another species of the genus, *Bulbostylis ciliatifolia*, occupies in the more extensive upper terrace sandhills. Many common xeric weeds occur in association with these species; these show no particular fidelity to the sandhills and are widespread throughout the state.

Roadside sites may vary from the hydric to conditions of extreme xericity. Here also the number of species found is quite extensive, composed for the most part of "camp follower" weeds of widespread occurrence. One species collected along a roadside and perhaps worth mention is *Paspalum notatum*. This grass was included in Blomquist's *The Grasses of North Carolina* (1948) on the basis of a "reported record near Wilmington." It is an introduced species.

SUMMARY

Collections of the vascular flora were made in a sandhills area where coarse Pleistocene sands overlie the interception of the Surrey Scarp with the Neuse River in southern Wayne and Lenoir counties. The area extends southward into the northern parts of Duplin County.

The following plant communities were observed: a xeric ridge community with *Pinus australis* as the dominant tree species in association with *Quercus laevis* and *Aristida stricta*; a pocosin community composed of elliptic-leaved evergreen ericads and hollies succeeded by members of the bay forest; an ecotonal community composed of members of both the adjoining ridge and pocosin communities as well as elements unique to the ecotone; and disturbed communities of cut-over pine woods, old fields, and roadsides.

All specimens collected have been deposited in the herbarium of the University of North Carolina. A total of 277 species were represented in the collections.

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SPECIES OF *CAREX* NEW TO THE FLORA OF NORTH OR SOUTH CAROLINA

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This the fourth of a series of papers on the distribution of the vascular plants in the Carolinas, in connection with the Flora of the Carolinas Project. Most of the records cited in this paper are from the collections of H. E. Ahles and other members of the project. Seven species of *Carex* are reported as new to South Carolina and five as new to North Carolina. Distribution data are based primarily on that given by Mackenzie, K. K. (1931. *Carex*, in N. Am. Fl. **18:** 1–168; 1935. *Carex*, in N. Am. Fl. **18:** 169–478) and Hermann, F. J. (1954. Am. Midl. Nat. **51:** 265–286). A few species not reported by Mackenzie and Hermann were found but are excluded on the basis of publications by Batson, W. T. (1953. The Flora of the Savannah River Project—1. Monocotyledons, Univ. S. C. Publ. Biol. **1:** 60–89) and Blomquist, H. L. and H. J. Oosting (1959. A Guide to the Spring and early summer Flora of Piedmont, North Carolina). All specimens are deposited in the University of North Carolina Herbarium unless otherwise noted.

The following species are new records to the state of South Carolina:

1. *Carex aestivalis* M. A. Curtis—Greenville Co.: dripping rocks, Hogback Mountain, 2500 ft., *D. C. Peattie 1255*. Pickens Co.: roadside, Sassafras Mountain, *Radford 16498*.

2. *C. leavenworthii* Dewey—Chester Co.: S. C. 9 at the Broad River, *O. M. Freeman 57128*.

3. *C. lupuliformis* Sartwell—Colleton Co.: swampy woods, 4.6 mi. ne. of Ritter near Chessey Creek, *Ahles 12207*.

4. *C. swanii* (Fernald) Mackenzie—Pickens Co.: rich wooded slope, 2.4 mi. s. of N. C.-S. C. state line on U. S. 178, *Ahles 14269*.

5. *C. virescens* Muhl.—Oconee Co.: dense moist woods, Lower Falls Whitewater River, *W. T. Batson 193* (Duke). Pickens Co.: moist woodland, 2.4 mi. s. of state line on U. S. 178, *Bell 3317*. The Oconee County specimen was determined by H. L. Blomquist.

6. *C. walteriana* Bailey—Jasper Co.: swampy ditch by S. C. 170, 1.2 mi. sw. of jct. with S. C. 46, *Bell 2404*. Williamsburg Co.: ditch, 4 mi. n. of Warsaw, *Radford 24802*.

7. *C. wildenowii* Schkuhr—Abbeville Co.: mixed deciduous forest, Mountain Creek, 6 mi. s. of Abbeville, *Radford 22801*.

The next five species are new to North Carolina:

8. *C. albursina* Sheldon—Ashe Co.: moist soil in wooded ravine on n. side of Big Tree Mountain on way to Johnsons Knob, *D. S. Correll 10875* (Duke). Madison Co.: woodland, 4 mi. n. of Walnut Creek, *Ahles 42626*. The Duke University specimen was determined by F. J. Hermann.

9. *C. jamesii* Schwein.—Northampton Co.: wooded slope, Roanoke River, 3 mi. w. of Boones Crossroads, *Ahles 41900*.

10. *C. tenax* Chapman—Wayne Co.: sandhill, 6.2 mi. nnw. of Seven Springs, *Radford 25550*.

11. *C. trichocarpa* Muhl.—Haywood Co.: dry road embankment, 2.6 mi. ene. of Crabtree, *Ahles 42183*.

12. *C. umbellata* Schkuhr—Avery Co.: roadside, 1 mi. ene. of Mitchell-Avery County line on U. S. 19E, *Ahles 39375*. Watauga Co.: wooded slope, 1.4 mi. ne. of Watauga-Avery County line on N. C. 603, *Ahles 39639*.

Carex albursina, *C. jamesii*, *C. trichocarpa* and *C. umbellata* represent southeastern extensions of range into North Carolina; *C. lupuliformis* and *C. swanii* represent southeastern extensions into South Carolina. *Carex tenax* is a southern species reported as far north as North Carolina for the first time. *Carex aestivalis*, *C. leavenworthii*, *C. virescens*, *C. walteriana* and *C. wildenowii* are within the general range of each species but have not been recorded from South Carolina prior to this report.

It is a pleasure to acknowledge the generous financial assistance given to the Flora of the Carolinas Project by Mrs. W. C. Coker, George R. Cooley, the Alumni Annual Giving Fund and the Smith Fund of the University of North Carolina, and the National Science Foundation in grant, G4450.

SPECIES NEW TO THE FLORA OF NORTH CAROLINA

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This is the third in a series of papers on distribution of vascular plants in the Carolinas in connection with the Flora of the Carolinas Project. Most of the records cited in this article are from the collections of the authors during 1958. Others are from the collections of C. R. Bell and O. M. Freeman, and a few noteworthy specimens were found in the local herbaria: University of North Carolina (NCU), North Carolina State College (NCSC), and Duke University (DUKE).

The inclusion of *Galium pedemontanum* All. is made to clarify a statement by Mark (1959) that Ahles had reported this species for the state. We presume 60 taxa (55 species and 5 varieties) enumerated in this report to be new records for the state of North Carolina. Two hundred and thirty-one taxa, including the 60 in this report, have now been published as records in North or South Carolina. The other two publications on distribution are: Rhodora **60:** 10–32 and the Journal of the Elisha Mitchell Scientific Society **75**(1): 35–43.

The family arrangement follows that of Fernald (1950); genera and species are listed alphabetically. Unless otherwise noted by symbol, the specimens are deposited in the University of North Carolina Herbarium.

It is a pleasure to acknowledge the generous financial assstance given to the support of the Flora of the Carolinas Project by Mrs. W. C. Coker; Mr. George R. Cooley; by the Alumni Annual Giving Fund and the Smith Fund of the University of North Carolina; and the National Science Foundation in grant, G4450.

Eragrostis curvula (Schrad.) Nees—Lenoir Co.: roadside, near U. S. 70, n. of Falling Creek, *Radford 31617*.

This species has been previously recorded only from Florida, Texas and Arizona by Chase (1950), and more recently South Carolina by Radford and Ahles (1959). It is a conspicuous grass, forming large clumps, and it is becoming more and more abundant particularly in South Carolina.

Sacciolepis indica (L.) Chase—Sampson Co.: boggy meadow, 1.8 mi. nnw. of Garland on N. C. 411, *Ahles 36795*. The specimen was determined by H. L. Blomquist.

This species, unlike the coarse *S. striata*, is very delicate and small and easily overlooked in the deeper vegetation. At the above cited locality it is quite abundant. Chase (1950) cites only, "Thomasville, Georgia." According to Dr. Blomquist he has seen specimens from Mississippi collected by R. B. Channell.

Tridens flavus var. **chapmani** (Small) Fernald and Griscom.—Dare Co.: maritime forest at Duck, *Radford 8772*. Richmond Co.: pine forest, Camp Mackall near Lumber River, *Radford 19269*. Robeson Co.: dry sandy slope, 2.8 mi. sse. of Lumberton along U. S. 74 at Lumber River, *R. F. Britt 2714*.

Fernald (1950), gives the range, under the name *Triodia chapmani* (Small) Bush, as, "Fla. to e. Tex., n. to se. Va., Cape May, N. J., Tenn. and s. Mo." Chase (1950) under the name *Tridens chapmani* (Small) Chase, shows a map from which North Carolina is excluded, although within the natural range.

Cyperus sesquiflorus (Torr.) Mattf. & Kukenth.—Bladen Co.: Brown Marsh Swamp, 2.9 mi. n. of Clarkton on road to Bluefield, *Ahles 33343*. Onslow Co.: railroad right-of-way, 1.8 mi. ne. of Jacksonville, *Ahles 35899*. Robeson Co.: pine-savannah, 1 mi. w. of Allenton on N. C. 211, *Ahles 33051*. Warren Co.: wet pasture, 0.7 mi. sw. of Eaton Ferry, *Bell 4372*.

Small (1933) describes this as a species of *Kyllinga*, giving the distribution as Florida to Mississippi and Georgia. Kukenthal (1936) considers this entity as a species of *Cyperus*. Radford & Ahles (1959) recorded this fragrant *Cyperus* from South Carolina.

Eleocharis erythropoda Steud.—Madison Co.: river margin, Hot Springs, *Freeman* (number and date missing); floodplain marsh on French Broad River, 2.5 mi. se. of Paint Creek, *J. G. Haesloop & Radford 7291*.

Fernald (1950) treats this species as *E. calva* Torr. with a range south to Virginia and Tennessee. Both Madison County specimens were found in one of the few limestone outcrop areas in the Carolinas.

Eleocharis obtusa var. **ellipsoidalis** Fern.—Ashe Co.: fresh-water marsh, 3.4 mi. ene. of Trout, *Radford 38682*.

Fernald (1950) gives the range of this well-marked variety as Massachusetts to southeastern Virginia. Svenson (1957) mentions the variety but does not give its range.

Fimbristylis miliacea (L.) Vahl—Harnett Co.: pond margin, 2.3 mi. s. of Olivia, *Laing 499*. Mecklenberg Co.: marsh, 1.8 mi. e. of jct. of U. S. 29-74 and Catawba River on U. S. 29-74, *Ahles 48609*. Polk Co.: moist sand at Collinsville, *Freeman 56698*.

This native of India has been found on ballast in Pennsylvania; in Florida, Arkansas and California according to Svenson (1957) and in South Carolina, Radford & Ahles (1959).

Rhynchospora intermixta C. W. Wright—Brunswick Co.: savannah, Southport, *H. L. Blomquist 5654* (DUKE). Carteret Co.: low places in savannah between Morehead City and Beaufort, *H. L. Blomquist 11240A*. Columbus Co.: edge of savannah e. of Old Dock, *Blomquist & Correll 9457*. Hoke Co.: old pond bed, 0.6 mi. se. of McNeills Lake (south of Dundarrach), *Ahles 29612*.

This small sedge is known only from Florida to Texas according to Small (1933).

Rhynchospora tracyi Britton—New Hanover Co.: shallow pond, Cape Fear Peninsula, *R. K. Godfrey 4826* (DUKE); shallow sand pond, 2 mi. e. Wrightsboro, *E. O. Beal & C. E. DePoe 3882* (NCSC).

This southeastern species had not been previously collected north of Georgia on the Atlantic Coast, according to Small (1933).

Scirpus fontinalis Harper—Onslow Co.: low swampy woods, Grants Creek, nw. of Silverdale, *Ahles 24226*.

North Carolina is well within the range of this species but according to Cappel (1954) it has never been found in this state.

Zephyranthes candida Herb.—Pamlico Co.: roadside, near Mesic. *Radford 42201*.

This South American native of the La Plata region is known from Florida to Texas according to Small (1933).

Salix purpurea L.—Madison Co.: stream bank, 0.1 mi. ne. of Whiterock, *Ahles 42589*.

Small (1933) says of this species, "E U. S." a range that could be interpreted to mean Maine to Florida, however, Fernald (1950) says, "Nfld. to Ont. and Wisc., s. to N. S., N. E. Va., W. Va., O., Ill. and Ia.", definitely excluding it from the range covered by Small (1933). Ball (1952) gives no range, merely stating, "sparingly escaped."

Polygonum sachalinense F. Schmidt—Avery Co.: roadbank, Montezuma Gap (elevation 3,943 ft.) 0.4 mi. e. of Montezuma, *Ahles 49649*. Mitchell Co.: wooded streambank, 2.7 mi. s. of Buladean on N. C. 26, *Ahles 47314*.

The range as given for this robust Asiatic species by Fernald (1950) is, "Mass. and N. Y. to Md." It forms large colonies as does *P. cuspidatum* and crowds just about everything else out. It is a very handsome plant, although, if it were to become common, it could certainly become an obnoxious weed.

Amaranthus tuberculatus (Moq.) Sauer—Mitchell Co.: railroad yard, Spruce Pine, *Ahles 49705*.

Under the name *Acnida altissima* Riddell, Fernald (1950) gives as the range for this species, "Ont. to Colo., s. to Ky., Ind., Ill. and Mo.; adv. eastw. to N. E." Gleason (1952) also as *Acnida altissima* has, "Ont. to Wis., Ky., and Col.; occasionally adventive eastward." Sauer (1955) cites only four states east of the Alleghenies; Connecticut, Massachusetts, Maine and New York. At the cited locality it was rare, but appeared to be well established.

Froelichia gracilis (Hook.) Moq.—Ashe Co.: railroad, West Jefferson, *Radford 41148*. Forsyth Co.: railroad right-of-way, 1.5 mi. nne. of Winston-Salem on old U. S. 311, *Ahles 51617*.

Fernald (1950) and Gleason (1952) give the range as south of Virginia in the east coast states. Ahles, Bell and Radford (1958) reported it for South Carolina. In the herbarium of the University of North Carolina there is also a specimen collected by J. A. Duke from Richmond County, Georgia. In Forsyth County it was very abundant spreading for miles along the railroad.

Mirabilis nyctaginea (Michx.) MacM.—Forsyth Co.: cinder bed along railroad, 0.9 mi. n. of Winston-Salem on old U. S. 311, *Ahles 45435*.

Small (1933) under the name *Allionia nyctaginea* Michx. gives the range as, "Ala. to Tex., Mont., and Wis." Fernald (1950) states, "Wisc. and Man. to Mont., s. to Ala., La. and Tex.; adv. and often weedy eastw. to Atl. States." Gleason (1952) under the name *Oxybaphus nyctagineus* (Michx.) Sweet says, "Man. to Ill. and La., w. to Mont. and Col.; now commonly established in waste places e. to Mass. and Va."

At the cited locality it was plentiful and very well established.

Dianthus barbatus L.—Wilkes Co.: orchard, 5 mi. e. of Moravian Falls, *Radford 11139*.

The range given by Fernald (1950) is Quebec to North Dakota south to New England, Delaware, etc. This European introduction seems to be well established at the Wilkes County locality.

Stellaria sylvatica (Beguinot) Maguire—Avery Co.: alluvial woods, 1 mi. ene. of Avery-Mitchell County line on U. S. 19E (s. of Ingalls), *Ahles 39384*. Haywood.: wooded slope, 6.7 mi. n. of Crabtree, *Ahles 38826*. Yancey Co.: wooded cove, 3.6 mi. nw. of Swiss. *Ahles 39081*.

Maguire (1952) states that this species is west of the Appalachians south to Tennessee, which would exclude North Carolina. Small (1933) treated this as *Alsine tennesseensis* (C. Mohr) Small in part.

Cimicifuga rubifolia Kearney—Ashe Co.: mixed deciduous forest, 2.3 mi. s. of Bina, *Radford 41227*.

This very local species has been known only from the Tennessee River in eastern Tennessee, Small (1933). The Ashe County specimen is in the New River drainage basin.

Ranunculus ambigens S. Wats.—Bertie Co.: in ditch, 1.1 mi. n. of Chapanoke then 0.3 mi. w., *Ahles 44255* and *48040*; roadside ditch, 2.2 mi. s. of Chapanoke, *Ahles 44257*.

Benson (1948) gives the distribution on the Atlantic Coast south to Virginia and possibly in Georgia, but not in the Carolinas.

Ranunculus flabellaris Raf.—Edgecombe Co.: cypress pool, Swift Creek, 4 mi. e. of Battleboro, *Radford 32004*.

This species is known from the Atlantic Coast south to southeastern Virginia and in the Mississippi Valley south to Louisiana according to Benson (1948), but not in the Carolinas.

Clematis ligusticifolia Nutt.—Currituck Co.: thicket, 3 mi. n. of Waterlily, *Ahles 48210*.

Fernald (1950) describes the range as Manitoba to British Columbia south to western Missouri, New Mexico, Arizona, and California. This is the first southeastern record.

Akebia quinata (Houtt.) Decne.—Madison Co.: mountain side, 7.3 mi. se. of Trust on N. C. 63, *Ahles 42451*.

Fernald (1950) under *Akebia quinata* Dene. says, "tends to spread from cult. (Introd. from Asia)." Gleason (1952), says, "rarely escapes." Since neither Fernald nor Gleason give an actual range for this interesting member of the Lardizabalaceae and since there seems to be no reference to North Carolina in the literature it is here cited as new. It appears that this species could be a very obnoxious plant, as it covers trees and shrubs just as densely as does *Lonicera japonica*.

Diplotaxis muralis (L.) DC.—Yancey Co.: roadside, 1.8 mi. sw. of Burnsville on N. C. 197, *Ahles 42855*; 2.9 mi. sw. of Burnsville, *Ahles 42859*; disturbed roadside, Cane River on U. S. 19E (w. of Burnsville). *Ahles 50846*.

Small (1933) says, "waste places and ballast, usually about seaports, various provinces, La. to Ont., and N. S." Fernald (1950) says, "especially near the coast, Que. and Ont., s. to N. S., N. E., N. J., Pa., O., Mich., Ill. and Ia." Gleason (1952) says, "N. S. to N. J. and Pa.; Wis. to Minn. and Ia." In Yancey County it is very abundant, turning roadsides and fields into a yellow haze for miles around when in flower, particularly in the Cane River valley. As a weed, it would seem to have the potential of becoming a very serious pest. The absence of specimens in the local herbaria warrant its inclusion here.

Potentilla argentea L.—Madison Co.: on road shoulder, U. S. 70 on French Broad River, 5 mi. e. of Marshall, *W. B. Fox* and *J. H. Beaman 4788* (NCS); roadside, 0.4 mi. nw. of Buncombe-Madison County line on U. S. 25-70, *Ahles 46492*. Swain Co.: open woods, Black Camp Gap, *Mary Caughey 707*. Wilson Co.: waste place, Black Creek, *Radford 33189*.

Gleason (1952) has reported this European weed as far south as Virginia.

Potentilla intermedia L.—Transylvania Co.: roadside weed at Rosman, *Freeman 57606*.

Fernald (1950) and Gleason (1952) give the range of this introduction south to Virginia. Small (1933) does not include the species. This is a southern range extension to within ten miles of the South Carolina border.

Prunus mahaleb L.—Mitchell Co.: wooded creek bottom, 0.8 mi. ne. of Hawk, *Ahles 43270*.

Fernald (1952) says, "N. E. to s. Ont. and southw." At the above locality only one small fruiting specimen was found.

Lotus corniculatus L.—Northampton Co.: waste ground, Conway on U. S. 158, *Ahles 45769*. Specimen was determined by R. L. Wilbur.

Small (1933) does not give a range, merely stating, "Waste-places and ballast, Coastal Plain, Atlantic and Gulf States," Fernald (1950)

says, "Nfld. to Minn., s. to Va. and O." Gleason (1952) says, "N. S. to O. and Va., especially in N. Y." At the above cited locality it was very scarce, represented by not more than a half dozen plants.

Melilotus indica (L.) All.—Robeson Co.: railroad right-of-way, Lumberton, *Ahles 23713*. Specimen was determined by R. L. Wilbur.

Small (1933) says, "Fla. to Tex., and locally northward to E. Canada." Fernald (1950) says, "southw., n. locally to N. S. and Minn." Gleason (1952) says, "abundant in the s. and Pacific states and adventive in our range as far n. as Vt." Although the ranges as quoted here would include North Carolina, the absence of a specimen or a published report for the state warrants its inclusion as new.

Trifolium hirtum All.—Orange Co.: campus, University of North Carolina, Chapel Hill, *Ahles 52709*.

Fernald (1950) says, "Prince Edward Co., Va." Gleason (1952) says, "Va." In Chapel Hill it is a weed in a few lawns.

Trifolium lappaceum L.—Columbus Co.: railroad yard and waste ground, Hallsboro, *Bell 12817*.

According to Small (1933) the range is, "Ala. and Miss. Nat. of Mediterranean reg."

Polygala grandiflora Walt.—Richmond Co.: ditch, 2 mi. sw. of Marston near U. S. 1-N. C. 77 jct., *Radford 19293*. Scotland Co.: sand hill, 2.5 mi. s. of Hoke-Scotland Co. line on U. S. 15–501, *Ahles 28626*; longleaf pine woods, 2 mi. sse. of Hoffman, *O. W. Gupton 1679*; longleaf-turkey-oak-wire grass association, 4.6 mi. sse. of Hoffman, *O. W. Gupton 1728*.

Small (1933) under the name *Asemeia grandiflora* (Walt.) Small, gives the range as, "Fla. to Miss. and S. C." In North Carolina it appears as a native plant.

Acalypha ostryaefolia Riddell—Durham Co.: edge of garden, low ground, 922 Demerius St., Durham, *H. L. Blomquist 16777* (DUKE & NCSC). Union Co.: railroad yard, Monroe, *Ahles 34087*.

Although the range of this species would seem to include North Carolina, the lack of specimens and published reports for the state seems to warrant this entry. Furthermore the statement by Fernald (1940), "*Acalypha ostryaefolia*, very distinct and almost handsome, abounds on steep weedy slopes near the Appomattox but along the Norfolk and Western [railroads], where it might have been indigenous, but too doubtfully so, since, except for a somewhat shaky record for New Jersey, it is otherwise known in the Atlantic States from South Carolina southward, primarily as a weed;" definitely excludes North Carolina. In Monroe it is very abundant and well established.

Croton monanthogynus Michx.—Cabarrus Co.: railroad, Harrisburg, *Ahles 16018*. Union Co.: strawberry field, 2.1 mi. sse. of Waxhaw, *Ahles 31389*; black locust planting, 1.3 mi. ne. of jct. U. S. 74-601 & N. C. 200 on road to Fairfield, *Ahles 34099*.

Although the ranges given for this species could include North Carolina, the absence of any mention ot it in local floras and its extreme rarity in the state warrant this report. There was only one plant found at the Cabarrus County locality, but it was a very abundant, gregarious weed at the two stations in Union County.

Phyllanthus niruri L.—New Hanover Co.: weedy roadside at jct. of U. S. 421 & State Docks Road, Wilmington, *Bell 16010*.

This species was recorded by Small (1933) only from Florida. Ahles, Bell and Radford (1958) reported it from South Carolina.

Melochia corchorifolia L.—Bladen Co.: low field, 2.9 mi. e. of Kelly on N. C. 53 near jct. of N. C. 210, *Ahles 37461*. Duplin Co.: cultivated field, 0.3 mi. nw. of Pin Hook, *Ahles 33166 & 35847*. There is also a specimen at NCSC with very incomplete label data, "Southeastern N. C., October 1958, *J. Hardin*".

Anoda cristata (L.) Schlecht.—Mecklenburg Co.: waste ground, Charlotte, *Ahles 50200*.

Fernald (1950) says, "se. Pa.; Ia. and Mo. to Tex." It is an adventive from southwestern United States and Mexico and may be only appearing here as a waif. It was not abundant, being represented by less than a dozen plants.

Hibiscus lasiocarpus Cav.—Northampton Co.: bog, Vultare on N. C. 46, *Ahles 45619* and *48473*.

Small (1933) says, "Ga. to Tex., Mo., and Ill." Fernald (1950) under *H. lasiocarpos* Cav. gives, "Ga. to Tex., northw. in Miss. basin to Ky., Ind., Ill., and Mo." Gleason (1952) says, "s. Ind. to s. Mo., s. to Fla. and Tex." In the bog at Vultare it was quite abundant.

Viola brittoniana var. **pectinata** (Bickn.) Alexander—Brunswick Co.: margin of mixed woods, 1.7 mi. ne. of Exum, *Bell 11599*. Gates Co.: flat pine-oak woods, 3 mi. sw. of jct. U. S. 13 & N. C. 37 on U. S. 13 (nw. of Gates), *Ahles*

44742; flat pine-oak woods, 1 mi. w. of Gates, *Ahles 48377*. Richmond Co.: creek bank, 4.6 mi. ese. of Ellerbee on Rocky Fork Creek, *Ahles 52696* (with *V. brittoniana* and *V. primulifolia*); low pine-oak woods, 4.2 mi. ese. of Ellerbee, *Ahles 52919*.

Fernald (1950), under the name *Viola pectinata* Bickn. says, "Mass. to Va." Alexander (1952) says, "Mass. to N. J., always occurring with the species." At the Gates County locality, where the plants were very abundant, *V. brittoniana* var. *brittoniana* was not seen in or near the colony.

Epilobium adenocaulon Haussk.—Avery Co.: wooded stream, 2.8 mi. nne. of Linville on N. C. 105, *Ahles 49526*. Mitchell Co.: roadbank, 1.2 mi. ese. of Carvers Gap on N. C. 261, *Ahles 49894*.

Gleason (1952) gives the distribution of this northern species as far south as Virginia.

Oenothera laciniata var. **grandiflora** (S. Wats.) Robins.—Harnett Co.: roadside, 1.7 mi. w. of Dunn on U. S. 21, *Ahles 52663*.

Fernald (1950) reports this variety from Texas, New Mexico and northeastern Mexico northeast to Missouri and Kansas. The Harnett County specimen is apparently a very recent introduction.

Aegopodium podagraria L.—Avery Co.: border of bog, jct. of U. S. 19E and N. C. 194 (between Elk Park and Heafton), *Ahles 43583*.

Rodgers (1950) reported this species from Forsyth County but the label data definitely indicated that the specimen was cultivated. Apparently the Avery County plant is the first known without cultivation in this state.

Angelica atropurpurea L.—Haywood Co.: river bottom, 3.6 mi. nw. of Crabtree on road paralleling Pigeon River, *Ahles 46666*.

This species was not reported from the southeastern United States by Rodgers (1950). Gleason (1952) gives the range of this northern species as far south as West Virginia and Indiana.

Buddleia davidi Franch.—Haywood Co.: cinder bed along railroad, 1.9 mi. e. of Canton on U. S. 19-23, *Ahles 46516*.

Fernald (1950) says, "becoming natzd. n. to Md. (Introd. from China)." At the cited locality it is not common, but seems to be well established.

Vinca rosea L.—Cumberland Co.: very abundant on waste ground, 2.3 mi. s. of Autryville and 2.9 mi. se on N. C. 210, *Ahles 36710*.

Small (1933), under *Catharanthus roseus* (L.) G. Don, gives the range as "Pen. Fla. and the Keys." In North Carolina it is commonly cultivated and frequently found as a waif, but rarely becomes established. At the above locality it was very abundant and apparently very well established. The owner of the property on which it occurred said it has been there for at least twelve years.

Ipomoea trichocarpa Ell.—Onslow Co.: brackish marsh, White River, Swansboro on N. C. 24, *Ahles 36041*.

Small (1933) says, "Fla. to Tex. and S. C." *Ipomoea trifida* G. Don is here considered conspecific with *I. trichocarpa*. (See Ahles in this issue of the Elisha Mitchell Journal, *Ipomoea trichocarpa* Ell. and *I. trifida* G. Don.)

Plagiobothrys hirtus var. **figuratus** (Piper) I. M. Johnston—Durham Co.: fallow fields, 2 mi. w. of Bland, *Ahles 23959*.

Jones and Fuller (1955) report this western species as recently adventive in Massachusetts, Illinois, Wisconsin, North Dakota, and South Dakota. This is the first record from the southeastern United States.

Verbena tenuisecta Briquet—Columbus Co.: sandy open soil, 5 mi. se. of Whiteville, *D. S. Correll & H. L. Blomquist 7341* (DUKE); wet roadside ditch by bridge over swamp creek, 0.8 mi. n. of Ironhill, *Bell 12733*. Cumberland Co.: roadside, 7 mi. ese. of jct. N. C. 210 and 53 on N. C. 210 (south of Autryville), *Ahles 24460*. Duplin Co.: roadside, 0.5 mi. s. of Beulaville on N. C. 41, *Ahles 24010*. Hoke Co.: sandy roadside, 0.8 mi. ne. of Hoke-Scotland Co. line on U. S. 15A (sw. of Bowmore), *Ahles 25065*. Robeson Co.: railroad right-of-way, Lumberton, *Ahles 23705*. Scotland Co.: occasional along weedy border of field about 3 mi. ne. of Laurel Hill towards Wagram, *R. L. Wilbur 5748* (DUKE); roadside, 0.5 mi. sw. of Silver Hill at mill pond on Jordans Creek, *Ahles 24790*.

Small (1933), under *Glandularia tenuisecta* (Briq.) Small, gives the range of this species as, "Fla. and Ga. to La." Moldenke (1949) excludes this species from North Carolina. Fernald (1950) does not include this species. Moldenke (1952) says, "naturalized from S. C. to Fla. and w. to Mo., Ark., La., Tex., and s. Cal." In southeastern North Carolina it is becoming very common on the sandy soil of the coastal plain and its showy blossoms are a welcome addition on the weedy roadsides.

Trichostema setaceum Houtt.—Alexander Co.: thin soil on granite exposure, Rocky Face Mt. n.

of Hiddenite, *W. B. Fox & R. K. Godfrey 2058* (NCSC). Specimen was determined by H. Lewis, 1958. Cleveland Co.: wooded roadside, Broad River about 4.5 mi. s. of Boiling Springs on N. C. 150, *Ahles 19180*. Cumberland Co.: sandy upland pine-oak woods, 2.3 mi. sw. of Hope Mills, *Ahles 36593*. Durham Co.: Co. road 560, 0.2 mi. n. of Eno River, *W. T. Batson, Jr., 1205* (DUKE). Forsyth Co.: field, Winston-Salem, *P. O. Schallert 6292* (DUKE). Granville Co.: on U. S. 15, 1 mi. n. of Tar River, *W. T. Batson, Jr. 1204* (DUKE). Madison Co.: bluff, 3.6 mi. nw. of Hot Springs on north side of French Broad River, *Ahles 50650*.

Although North Carolina is within the natural range of this species according to the manuals, a map published by Lewis (1945) warrants its inclusion here. Nowhere on the map or in the citation of specimens are North Carolina records mentioned.

Mazus miquelii Makino—Durham Co.: escaped and apparently thriving along creek above and below Duke Garden, *W. T. Batson, Jr. 216* (DUKE). This specimen is labeled *M. japonicus*; lawn weed in Duke Gardens, *Bell 5922*. (This specimen is labeled *M. reptans*).

This species is not listed in any of the eastern North American manuals, however it is believed that the *Mazus japonicus* (Thundb.) Ktze. in Blomquist & Oosting (1953) is probably this species. This is the common species, cultivated in gardens and treated by Bailey (1949) as *M. reptans* N. E. Br., a name not treated by Index Kewensis. The plants spread rapidly in cultivation and it is not surprising therefore to find it as an escape. The species was probably planted at one time at Duke University and our specimens may represent merely a remnant of cultivation rather than an escape.

Galium mollugo L.—Alleghany Co.: roadside, Sparta, *Radford 34086*. Ashe Co.: waste place, West Jefferson, *Radford 32865*. Avery Co.: roadbank, 1.3 mi. w. of Banners Elk on N. C. 194, *Ahles 43589*. Watauga Co.: road bank, 0.7 mi. e. of Laxon on U. S. 421–221, *Ahles 43942*.

According to Fernald (1950) and Gleason (1952) this European plant is naturalized in the northeastern United States as far south as Virginia.

Galium pedemontanum All.—Haywood Co.: road bank, 5.2 mi. n. of Crabtree on N. C. 209, *Ahles 42196*. Orange Co.: lawn weed, Chapel Hill, *Ahles 25189*.

Fernald (1950) and Gleason (1952) give the range as, "W. Va. and Ky." Several years ago it was collected by H. E. Ahles in Missouri and Illinois. Mark (1959) reports this species from Max Patch Mountain in North Carolina with the comment that Ahles previously reported it for the state. This error was due to a statement made at the time Ahles identified Mark's specimen with the comment that Ahles had a specimen of it that we planned to publish on: however, we did not get to do it before it was published by Mark. In Chapel Hill, this species is a rather rare lawn weed, but in Haywood County it is very abundant on the road bank, stream bank and around rock outcrops in pastures. It is not a serious weed since it is a winter annual and soon disappears.

Echinocystis lobata (Michx.) T. & G.—Watauga Co.: river bottom, New River, 3.2 mi. sw. of Todd, *Ahles 47704*.

Fernald (1950) says, "N. B. to Sask., s. to Fla. and Tex." The scarcity and lack of herbarium sheets warrants its inclusion here as new to the state. At the cited locality only a few plants were seen.

Wahlenbergia marginata (Thunb.) A. DC.—Robeson Co.: roadside in sand hills, 0.8 mi. sw. of jct. U. S. 74 & 301 (w. of Lumberton), *Ahles 28850*.

Ahles, Bell and Radford (1958), record this species under the name *W. gracilis* Schrad. as new to South Carolina, recording seven counties. While collecting in 1957 in southeastern North Carolina great effort was made to find this species. On June 21, it was found very abundant at the cited locality. This however, was the only colony found in the state. The recording of this species previously for South Carolina under the name *W. gracilis* Schrad. was due to an over-sight of McVaugh's publication (1943–44).

Crepis japonica (L.) Benth.—Pasquotank Co.: roadside, 1.3 mi. n. of Knobbs Creek on U. S. 17-158 then 0.1 mi. e. (n. of Elizabeth City), *Ahles 40082*.

Small (1933) says, "S. La. Nat. of Japan." Fernald (1950) says, "Pa. to Va." It was fairly abundant at this locality and very well established.

Eupatorium resinosum Torr.—Bladen Co.: bog near Ammon, *L. Melvin 5202*. Hoke Co.: bog, 1.7 mi. w. of Hoke-Cumberland Co. line on road from Overhills to Lobelia, *Ahles 36508*.

Fernald (1950) gives for the range of this species, "N. J. and Del." and Cronquist (1952) gives

the same range but adding, "also collected a century ago on L. I." The Bladen and Hoke County specimens give quite an extension of range southward particularly since both these counties are in southern North Carolina.

Eupatorium saltuense Fern.—Caswell Co.: Stony Creek Mountain, *J. A. Duke 0403*. Granville Co.: upland woods border, 0.7 mi. w. and 0.6 mi. s. of Culbreth, *Ahles 17535*. Warren Co.: upland woods border, 0.3 mi. ne. of jct. of N. C. 58 & U. S. 158A, *Bell 4308*.

This recently described species has been previously recorded only from southeastern Virginia by Fernald (1950).

Hypochaeris elata (Wedd.) Griseb.—Johnston Co.: railroad, Princeton, *Radford 25330*.

Monachino (1957) cites two specimens of this species, one from Georgia and the other from Allendale County, South Carolina. Monachino points out that the plant treated by Small (1933) under the name *Crepis foetida* L. is probably this species since the specimens labeled by Small as such belong here. In addition to the specimen from Allendale County, South Carolina, collected for the Flora Project and reported on by Monachino, we can add the following 19 counties for that state: Bamberg, Beaufort, Berkeley, Calhoun, Charleston, Cherokee, Clarendon, Dillon, Dorchester, Edgefield, Fairfield, Georgetown, Hampton, Lee, Lexington, Marion, Marlboro, McCormick, and Williamsburg. These counties, when plotted on a distribution map, give the impression that it is found throughout the lower Piedmont and Coastal Plain of South Carolina. In North Carolina it is known only from the one station.

Solidago leavenworthii Ell.—Cumberland Co.: upland woods, 1 mi. n. of Beard, then 0.7 mi. wnw., *Ahles 33551*. Robeson Co.: dried-up weedy savannah, 3.2 mi. se. of Red Springs on N. C. 211, *Ahles 33001*.

Small (1933) says, "Fla. to S. Car." At neither locality was this plant abundant. It bears a striking resemblance to a very narrow leaved, small edition of *Solidago gigantea*.

Sonchus uliginosus Bieb.—Haywood Co.: cinder bed along railroad, 1.9 mi. e. of Canton on U. S. 19–23, *Ahles 46520*.

Fernald (1950) says, "N. B. to Mich., s. to s. N. E., Del., Md., O., Ind. and Ill., and probably of wider range." At the cited locality the plants covered an area of about six square feet. It was the only colony observed.

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THE EFFECT OF PLANTING DATES ON INFESTATION OF THE CARROT RUST FLY, (*PSILA ROSAE*) (F.) (PSILIDAE: DIPTERA)[1]

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Introduction

Late planting, alone or combined with early harvesting, has been recommended by many entomologists over the years for the control of the carrot rust fly. In 1948, a survey of the Bradford Marshes was made to determine the use made of such timing in the area, and whether its use could be improved upon in the future. From 1949 to 1952, field experiments were conducted to determine the best time of planting to avoid injury.

Review of Literature

Whitcomb (1938) reported that carrots planted early in the season were more susceptible to attack by the first generation than those planted later; that the later carrots were planted the more subject they were to second-generation attack. Glendenning (1946), in British Columbia, stated that: "In locations where the appearance of the flies is regular or can be determined, seasonal sowing is a satisfactory means of avoiding infestation. For instance, early carrots sown in April or before, will be infested by the first-generation flies; but if seeding is delayed until early May, the seedlings will avoid infestation by this generation, will not require treatment and can be harvested in early July, before the second generation appears." Hanson and Webster (1941) stated that the fall crop of carrots when planted early in the spring, furnished food for the pest throughout the entire season. They point out that even though the first brood of maggots may not cause severe injury in a field, flies resulting from these maggots deposit their eggs close to the place of emergence, and a much larger second brood develops in the same field. For this reason, late planting of the main crop serves to reduce damage. By practically eliminating first-brood injury in a carrot field, injury by the second-brood larvae late in the season is minimized. However, the value of late planting is negligible if early plantings are grown in close proximity, permitting migration from one planting to another.

Brown (1954) surveyed damage caused to carrots in England and concluded that the later the planting the less damage, especially in commercial plantings.

Methods

Survey.—Thirteen fields of Nantes-type carrots were examined. Planting dates varied from April 10 to July 18. The percentage of injured roots at harvest was the criterion used for evaluating the planting dates. Samples consisted of 100 carrots pulled at random from each field.

Field Experiments.—The experimental design was a randomized block replicated four times. A plot consisted of three rows each 100 feet long. There were ten planting dates: carrots were planted weekly from May 16 to July 18. The percentage of injured carrots at harvest and the weight of roots were the criteria used for evaluating the planting dates. Samples consisting of 200 carrots from each plot were harvested October 4.

Results

Survey.—Table I shows that carrots planted from May 12 to June 5 were the most severely infested; from April 10 to 27, and from June 8 to 18, the least severely infested.

The survey revealed that almost no use of timed planting is made at the Bradford Marshes. Because of cropping practices, its use would be limited. However, a combination of timed planting and harvesting could be used to reduce infestations slightly, although not to eliminate them.

Bradford conditions and agricultural practices are unique, and may be explained as follows: two crops of carrots and celery are grown annually, an early and a late crop. These two crops supply food for larvae of the first and second generations of the insect respectively. The early

[1] The work reported herein was carried out in partial fulfilment for the degree of Doctor of Philosophy, Cornell University, Ithaca, N. Y. while the author was employed by the Canada Department of Agriculture, Ottawa, Ontario, and the Ontario Agricultural College, Guelph, Ontario.

TABLE I

Planting date survey of carrots, Bradford, Ont. 1948

Grower	Date Planted	Date Harvested	Percent Infested
Davis Brothers	April 10	July 28	1
Winter Brothers	20	28	19
P. Klassen	20	27	8
A. van Dyke	21	27	4
N. Becker	25	17	9
P. Klassen	27	27	13
F. Doane	May 12	17	74
J. Wist	29	29	30
J. Wist	June 5	Sept. 17	24
P. Klassen	8	17	15
N. Becker	9	14	12
J. Wist	12	17	4
J. Wist	18	17	7

TABLE II

Infestation and yield of carrots, planting dates experiment, Archer Farm, Bradford, Ontario, 1952

Planting Date	Percent infested	Average weight, in pounds
May 16	42.5	16.2
23	44.0	26.2
30	46.2	20.7
June 6	45.7	22.7
13	46.1	6.7
20	41.1	12.7
27	41.5	8.7
July 4	41.0	6.5
11	41.2	5.5
18	37.6	3.7
L.S.D. 19:1	No significant	5.23
99:1	differences	7.07

crop is harvested before attaining its full growth, and sold as "bunched" carrots. The late crop is left in the ground until late fall, and sold as "bushelled" or storage carrots. The growing periods overlap so that two crops are never grown on the same land in one year. However, fields of early and late carrots are never very far apart, for the following reasons: (1) About two-thirds of the population, or about 500 growers, grow carrots; each grows about two acres. (2) The Marsh is an area of concentrated cultivation; 6,000 acres of the 7,000 acre total are cultivated. There are few natural barriers between properties, such as trees, heights of land, fence rows, or uncultivated areas. (3) In small holdings, it is often necessary to grow both early and late crops. Thus, host plants are plentiful and close together, and each generation of the insect has its own crop on which to feed.

Although carrots cannot be freed from the possibility of attack by one generation, it is possible to avoid attack by both generations.

Field Experiments.—In 1949, 1950, and 1951, infestations were less than two per cent; thus, no significant data were obtained. In 1952, a moderate infestation developed in the experimental field.

Table II shows the planting dates, the per cent of infested carrots, and the weights of the samples. The data show that carrots planted between May 16 and July 18 were heavily infested. Yield data, however, indicated that to get maximum yields carrots should be planted between May 16 and June 20. Carrots planted later produced few roots that were marketable either as "bunched" or "bushelled" carrots. In the Bradford Marshes the peak emergence of the carrot rust fly occurs about June 10 for the first generation; and August 20 for the second generation.

DISCUSSION

Data show that time of planting does not reduce infestation. However, at Bradford, early planting to allow early harvesting can be used to avoid some injury.

To get a satisfactory yield of early carrots, planting must be done at a time that will result in maximum exposure to infestation. However, if harvested early enough some injury can be avoided. The first second-instar larvae were found on July 10, 1952; thus, carrots could be harvested just before or soon after, this date, before many worms entered the carrots. This is about the earliest date that early carrots would be large enough to harvest, because they require about a month and a half of growing time. Thus, effectiveness of early harvesting would vary from year to year; in a poor growing year the carrots could not be pulled early enough to avoid injury. In a good growing season, much injury could be avoided.

Late carrots require about two months to attain full size. In 1952, the first second-instar larvae were found on September 11. Therefore, to be mature on that date, carrots would have to be planted on or before July 10. Growers prefer to delay carrot-harvest until as late as

November, harvesting instead crops that are more perishable and not intended for storage. This is probably a major reason why the carrot rust fly population is maintained. However, growers would probably practice early harvesting when necessary, i.e., in years of severe infestation. In years of lighter infestation, harvest might be delayed safely, especially if insecticides were used.

SUMMARY

All carrots planted weekly between May 16 and July 18, 1952, were infested to a similar degree. However, those planted after June 6 had decreased yields and those planted after June 27 were almost entirely unmarketable. Some reduction of infestation can be obtained by manipulation of planting dates, but frequently this is not practical. It is, however, a general practice in the Bradford Marshes to plant carrots early and sell them as "bunching" carrots; and to plant late and harvest in October or November for storage carrots. Thus taking advantage of knowledge of the seasonal history of the carrot rust fly and adapting the time of planting and harvest to it, the amount of crop loss may be reduced.

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VARIETAL RESPONSES OF CARROTS TO CARROT RUST FLY, *PSILA ROSAE* (F.) (DIPTERA: PSILIDAE)[1]

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Introduction

Varietal differences in damage to carrots by the carrot rust fly, *Psila rosae* (F.), have been reported by entomologists and by vegetable growers. In 1948, a survey was made at Bradford, Ont., to determine whether varietal differences occurred in this area. From 1948 to 1952, experiments were conducted to determine whether some varieties were more susceptible than others to carrot rust fly attack.

The carrot rust fly has been known in Canada since 1885, when Fletcher (1886) reared specimens from infested carrots purchased on the Ottawa market. Since that time the pest has been found in all the provinces of Canada except Alberta, Saskatchewan, and Manitoba.

This paper is a summary of work conducted at the Bradford Marshes located about 40 miles north of Toronto, Ont. According to growers, the first carrot rust fly damage to carrots grown in organic soil in the area was observed about 1936. It was not until the severe outbreaks of 1946 and 1947 that growers requested aid in controlling the pest. In 1947, chemical control experiments were conducted; further experiments were carried on each year up to, and including 1954. In 1948, studies on the biology of the insect were initiated.

Review of Literature

Ormerod (1879) reported that the French Horn varieties were less susceptible to attack than the long varieties. Fletcher (1900) quoted a Mr. Clifton, who wrote, "Of late years we have about abandoned the culture of the tender varieties on account of its [carrot rust fly] depredations. It does not trouble the hardier varieties here apparently, for we can get full crops of the white carrots, when the orange ones are a complete failure in the same field." Blair (1927) and Whitcomb (1938) indicate that white carrot varieties are as susceptible to attack as orange varieties. They report that the short, thick varieties are less seriously damaged than the long varieties. In Russia, Savzdarg (1927) found that in testing over 80 varieties, classified into three groups according to size and shape, short forcing types were most susceptible to carrot rust fly attack; half long types were moderately susceptible; and long types, least susceptible. Bremer et al. (1940) conducted experiments in Germany showing that Nantes-type varieties were most susceptible; French forcing type, namely Paris Market, least susceptible; and Scarlet Horn and Long Orange types, intermediate in susceptibility. Ahlberg (1944) stated that of the varieties tested in Sweden, some showed more attractiveness than others to the ovipositing females. He believed that some reduction in infestation to the main crop would result if the most attractive variety were planted as a trap crop. Lamb (1953) found that in New Zealand Osborne Park was the most susceptible variety to carrot rust fly attack, Manchester Table the least susceptible and Chantenay, Early Horn and James Intermediate moderately susceptible.

Classification of the Orange-fleshed Varieties of Carrots

During the years 1948, 1949, and 1950, all the carrot varieties, regardless of classification, grown in the Bradford area were included in the varietal susceptibility tests. The varieties tested in 1951 and 1952 were selected according to the method of Babb, Kraus, and Magruder (1950). These workers classified carrots in nine major groups based on such characteristics as root size and shape, color of the shoulder, and size and degree of the indentation of the collar. The seven varieties observed at Bradford during the years 1948 to 1952 belonged in the following four major groups:

Danvers Type: Danvers Half Long
Imperator Type: Imperator

[1] The work reported herein was carried out in partial fulfilment for the degree of Doctor of Philosophy, Cornell University, Ithaca, N. Y. while the author was employed by the Canada Department of Agriculture, Ottawa, Ontario and the Ontario Agricultural College, Guelph, Ontario.

TABLE I

Varietal infestation data from fifteen fields surveyed in 1948, Bradford, Ontario

Variety of Carrots	Number of Fields Examined		Average per cent infestation	
	First generation	Second generation	First generation	Second generation
Scarlet Nantes	5	5	12.6	12.4
Imperator	2	1	10.0	2.0
Strong Top Nantes	2	—	10.5	—
Touchon	1	—	74.0	—

Nantes Type: Scarlet Nantes
Touchon
Amsterdam
Strong Top Nantes
Chantenay Type: Red Cored Chantenay

Methods and Results

During 1948 sixteen fields chosen at random in the Bradford Marshes were examined and a sample of 100 carrots was taken from each for infestation records. Ten fields were infested by the first, and six by the second generation of the pest. Varietal and infestation data from this survey are shown in Table I.

All the experiments consisted of randomized blocks replicated three times in 1948, 1949, and 1950; five times in 1951; and four times in 1952. A sample of 100 carrots was taken from the center rows of each plot in 1948, 1949 and 1952; and of 200, in 1950 and 1951. Each sample was washed, drained and examined for carrot rust fly injury. The criterion of susceptibility to the carrot rust fly was the percentage of roots injured to any extent at harvest. Data from the 1948, 1950 and 1952 varietal susceptibility trials infested by the first generation of the insect and for the 1949 to 1952 trials, infested by the second generation, are summarized in Table II.

In 1952 the infested carrots from the samples of each variety were examined and the severity of damage recorded. The data obtained are shown in Table III. Slight, medium and severe injury categories were set up arbitrarily; slight injury when the apical third of the root was damaged, medium injury when the middle and apical third was damaged and severe injury when damage occurred on all three portions of the carrot root.

The 1948 survey results as shown in Table I indicated that there might be some varietal susceptibility.

TABLE II

Varietal differences in the amount of carrot rust fly injury to carrots 1948–1952, Bradford, Ontario

Variety	Percent of carrots injured per sample						
	First generation			Second generation			
	1948	1950	1952	1949	1950	1951	1952
Imperator	1.5	1.7	2.0	0.7	0.0	24.0	78.2
Touchon	6.2	1.0	7.2	1.3	0.3	—	79.2
Red Cored Chantenay	2.2	0.8	0.7	0.0	0.0	26.7	85.7
Amsterdam	1.7	0.5	5.2	—	0.5	32.5	87.2
Danver's Half-long	2.2	0.3	3.7	2.3	0.0	32.1	91.2
Scarlet Nantes	—	0.5	5.2	2.0	0.2	51.2	91.5
L.S.D. 19:1	—	—	—	—	—	6.8	4.7
L.S.D. 99:1	—	—	—	—	—	9.3	6.4

TABLE III

Severity of carrot rust fly injury to carrot varieties 1952, Bradford, Ontario

Variety of Carrots	Percent carrots injured		
	Slight	Medium	Severe
Imperator	69.6	23.6	6.8
Red Cored Chantenay	51.9	32.7	15.4
Danver's Half-long	38.2	30.9	30.9
Touchon	53.4	8.4	38.2
Amsterdam	33.2	21.2	45.6
Scarlet Nantes	25.5	27.0	47.5

In Table II it will be noted that only the second generation data for 1951 and 1952 show significant differences. In 1951 Imperator was significantly more resistant to attack than Amsterdam, Danver's Half-long and Scarlet Nantes. In 1952 Imperator and Touchon were significantly resistant when compared with Red Cored Chantenay, Amsterdam, Danver's Half-long and Scarlet Nantes.

In 1952 Scarlet Nantes carrots had the largest number of severely infested roots; Imperator the least. The other varieties were intermediate as shown in Table III.

Discussion

Although the results of the experiments from 1948–1950 and the first generation in 1952 did

not show any significant differences, the trend indicated that Scarlet Nantes carrots, or at least varieties of the Nantes-type, were more susceptible to carrot rust fly attack than other types, especially Imperators. In 1952 Nantes-type varieties were most severely attacked whereas Imperator-type varieties were least severely attacked. The other varieties in the experiment were intermediate. The data on varietal responses to carrot rust fly attack presented here are by no means conclusive for all areas but appear to be true for the Bradford Marshes.

SUMMARY

Six varieties of carrots were tested for resistance to carrot rust fly in the Bradford area 1948–1952. One hundred or more carrots from each plot of each variety were washed, drained and examined for injury at time of harvest. All varieties tested were subject to carrot rust fly attack. However, varieties of the Nantes-type were most susceptible, Danver and Chantenay intermediate, and the Imperator was least susceptible. In areas where carrot rust fly causes damage, choosing the least susceptible variety would reduce the loss by this pest.

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SOME EFFECTS OF TEMPERATURE AND TURBULENCE ON THE EXTERNAL MORPHOLOGY OF *CYCLOPS CAROLINIANUS*

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Introduction

During development, cyclopoid copepods pass through 6 nauplius stages and 6 copepodid stages; each stage except the last is terminated by a moult. The last, or 6th copepodid, stage is a fixed stage of maturity with an armature of spines and setae that is unchanging. For some species armature is practically invariable, regardless of the environmental conditions, but in certain species the armature of spines on the terminal segments of the exopods of the swimming feet shows notable flexibility and responsiveness to environmental conditions attending the development of the copepod.

Investigators (Coker, 1924a & b, Aycock, 1942) have showed that examples of *Cyclops vernalis* Fischer reared from the early nauplius stages to adults in vessels of water of low temperatures will generally have more spines on the terminal segments of the exopods of the swimming feet than those reared in vessels of warmer water. These segments are armed with setae and spines. A seta is a long, slim, flexible structure generally bearing lateral hairs in two series, whereas, a spine is a short, stiff structure often bearing two rows of spinules. The number of setae for a particular segment is constant, unless some are replaced by spines (as in the case of some endopod segments), but the number of spines is independently variable within limits in certain species of copepods. Variations in number of spines of terminal segments of exopods of the swimming feet are due to additions or to non-development of spines and are rarely if ever due to substitution of spines for setae. On the other segments of the feet, particularly on the terminal segments of endopods of the second, third and fourth feet, spines may replace setae. It is convenient to show the number of these spines on end segments of exopods by formulas. The minimum spine formula is usually 2,3,3,3 for legs on both right and left sides; the first swimming foot having two spines on the end segment of its outer ramus, the second foot three spines, etc. (see figs. 1–4). The maximum spine formula is usually 3,4,4,4 for legs of both right and left sides (see figs. 5–8). Rarely there are supernumerary spines above the maximum or a deficiency below the minimum. Often the formula for one side may differ from that of the other side. For example, we may find:

$$\frac{\text{Right}}{\text{Left}} : \frac{2,3,4,4}{2,4,4,4} \quad \text{or} \quad \frac{2,3,4,3}{3,4,4,3}, \text{ etc.}$$

The maximum formula is usually obtained when rearing copepods from the earliest nauplius stages in water of 7° to 10°C. (Aycock, 1942—for *C. vernalis*). The minimum formula is not so regularly obtained by rearing the animals at higher temperatures (28° to 30°C.). A reduction in number of spines generally occurs, but sometimes even a maximum formula is encountered at such temperatures and often we find a mixed formula such as $\frac{\text{R. } 2,3,4,3}{\text{L. } 2,3,3,4}$. This is in accord with the earlier experimental results obtained from a small number of *C. vernalis* by Coker and Sheppard in 1936 (unpublished manuscript).

It was also found by Aycock (1942) that American *C. vernalis* reared in "warm" water (28.1°C.) are conspicuously smaller (average about 1.063 mm. for females) than those reared in "cold" water (7.7°C.) (average about 1.517 mm. for females). The caudal rami of the 28.1°C. examples were less slender (mean ratio of furcal width-length being 0.284 for females and 0.298 for males) than those of the 7.7°C. examples (mean ratio 0.217 for females and 0.207 for males). These figures are very close to those found by Coker (1953) in European examples he reared at low temperatures in Paris, France.

Many species of copepods show variability in size when reared at different temperatures; some species show variability in proportions of caudal rami and of segments of the swimming feet (see Yeatman, 1944, on *Cyclops viridis*—pages 21 and 22), but, as noted above, only a very few species have a variable spine number. Dr. R. E. Coker and Mrs. Alice Gottaas reared examples of *Eucyclops agilis* (Koch) under extreme differences

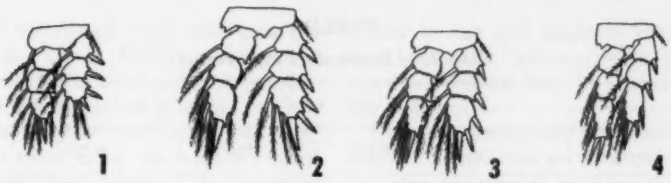

FIG. 1. First foot.
FIG. 2. Second foot.
FIG. 3. Third foot.
FIG. 4. Fourth foot.

FIGS. 1–4. Swimming feet of *Cyclops carolinianus* exhibiting the 2,3,3,3 spine formula of the terminal segment of exopods.

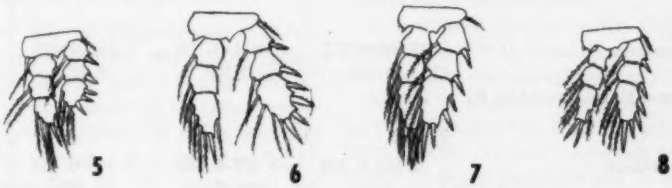

FIG. 5. First foot.
FIG. 6. Second foot.
FIG. 7. Third foot.
FIG. 8. Fourth foot.

FIGS. 5–8. Swimming feet of *Cyclops carolinianus* exhibiting the 3,4,4,4 spine formula of the terminal segment of exopods.

of temperature, but could never discover any change in spination.

Since the recently described species *Cyclops carolinianus* Yeatman (1944) closely resembles *C. vernalis*, but is generally smaller, and has also a variable spine formula in nature, it seemed desirable, as a preliminary study, to subject it to temperature experiments to determine if it reacts to temperature in the same way as does *C. vernalis*.

This report is based on a thesis submitted in 1952 to the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy. It is with pleasure that the writer expresses his gratitude to Professor R. E. Coker for constant encouragement and untiring assistance during the course of this investigation.

TEMPERATURE EXPERIMENTS

Material and Methods: C. carolinianus nauplii of known parentage were placed in 10 cc. shell vials which in turn were placed in a constant temperature apparatus (Coker and Constable, 1936). Some were placed in a chamber in which a temperature of 8.3°C. was maintained with little variance (less than 1°C.) and others were placed in a chamber of 28.7°C. Both sets of vials were kept replenished with supplies of unicellular green algae (chiefly Chlorella and Ankistrodesmus) and of *Paramoecium multimicronucleatum*, which experience has showed to be excellent food for many cyclopoid copepods. Filtered aquarium water with supplemental food was used for these and all other experiments.

Results of Temperature Experiments (see Table 1): *C. carolinianus* reacted like *C. vernalis*. Those placed in the low temperature chamber required 40 to 60 days to mature, were relatively large in size (males averaged about 0.82 mm. and females about 1.21 mm. in body length), and had the maximum spine formula. Those reared in the high temperature chamber required only about 12 to 14 days to mature, were smaller in size (males about 0.64 mm. and females about 0.84 mm. in body length), had rather stubby appendages and caudal rami, and usually had the minimum spine formula.

Copepods with the minimum spine formula, $\frac{\text{R. } 2,3,3,3}{\text{L. } 2,3,3,3}$, have a total of 22 spines on the foot segments in question; those with the maximum formula, $\frac{\text{R. } 3,4,4,4}{\text{L. } 3,4,4,4}$, have a total of 30 spines on these segments; whereas animals with irregular spine formulas have, generally, spine totals ranging between 23 and 29, inclusive. After

TABLE 1

Results of temperature experiments

Temperature	8.3° C.		28.7° C.	
Sex & Numbers	57 ♀♀	98 ♂♂	76 ♀♀	124 ♂♂
Body length in mm.				
High	1.50	0.90	0.90	0.69
Low	0.95	0.75	0.80	0.60
Average	1.21	0.82	0.84	0.64
Caudal ramus $\frac{\text{Width}}{\text{Length}}$				
Limits of variance	0.200–0.177	0.214–0.176	0.262–0.221	0.310–0.220
Average	0.194	0.198	0.227	0.270
Terminal segment of endopod of P_4 $\frac{\text{Width}}{\text{Length}}$				
Limits of variance	0.413–0.360	0.407–0.361	0.460–0.368	0.453–0.365
Average	0.374	0.371	0.447	0.407
Number of spines on exopod 3 of P_1–P_4 of Copepods	1,645	2,779	1,904	3,024
% increase in numbers of spines above minimum	85.7%	79.5%	38.2%	29.8%
Combining number of spines	4,424		4,928	
% increase above minimum	81.7%		33.0%	

the spine counts were made on the recovered adult copepods, the percentage of spines above the minimum was computed for copepods reared, respectively, at 8.3°C. and 28.7°C.

To compute this percentage, the following formula is used:

$$\frac{(s - 22n) \times 100}{n(30 - 22)}.$$

In this formula s represents the total number of spines counted, n the number of copepods; as previously mentioned, 22 is the normal minimum spine count for an individual copepod of this species, and 30 the normal maximum spine count. To illustrate, in one experiment 57 female copepods recovered from the 8.3°C. vessel had a combined count of 1645 spines on end segments of exopods. The formula was applied as follows:

$$\frac{(1645 - 22 \times 57) \times 100}{57 \times 8} = \frac{39100}{456} = 85.74\%$$

Details of the results are shown in Table 1. Fifty-seven female copepods reared at 8.3°C. showed an 85.7% increase in number of spines above the minimum as compared to 38.2% for the seventy-six 28.7°C. females. Ninety-eight males reared at 8.3°C. showed a 79.5% increase as compared to 29.8% for the one hundred and twenty-four warm water males. The combined males and females (155 from cold water) showed an 81.7% increase as compared to 33.0% for the 200 warm water males and females.

As used above and throughout this paper, the expression "increase in number of spines" refers to number of spines supplemental to normal minimum (22). That number forms the permanent armature of mature copepods, since spines are not added after maturity is reached.

Turbulence Experiments

The experiments of William Rowan (1938), in which the daily activity of caged juncos in dim light was increased by a travelling bar that swept the perch and floor at regular intervals, prompted me to investigate the effects, if any, of increased activity on the morphology of cyclopoid copepods. Shortly, Brooks' (1947) experiments on the effects of turbulence on cyclomorphosis in Daphnia gave new stimulus to the investigation.

Both *C. vernalis* and *C. carolinianus* are active

swimmers and not usually water-treaders as are *Mesocyclops edax* (S. A. Forbes), *Tropocyclops prasinus* (Fischer) and a few other cyclopoids. They swim from one resting place to another, actively pursue prey or each other, and may even creep or swim along the surface film of bodies of water. Since most of the copepods tended to settle on the bottom of the experimental containers, the problem was one of keeping the water stirred up from the bottom.

(a) Air-Caused Turbulence Experiments

Materials and Methods (Experiments I & II): Since no low-speed stirrers were immediately available, glass funnels (721 cc. and 100 cc. capacities) were first used as containers with turbulence secured by bubbling compressed air in at the bottom through a tiny hole in a plug of aquarium cement (see fig. 9). Compressed air effectively stirred up water and copepods, but added the factor of increased aeration in turbulence containers. The first experiments were mainly exploratory; should any effect on form follow turbulence produced in any manner, the need for further experimentation would be indicated.

During April and June 1947, first-stage nauplii of *C. carolinianus* were divided between experimental funnels with air-caused turbulence and double controls which consisted of (1) funnels similar in size and shape to the experimental ones, but with drainage stem plugged with aquarium cement, and (2) control vessels with flat bottoms.

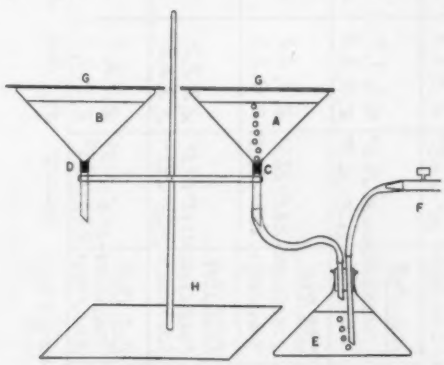

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FIG. 9. Air-caused turbulence apparatus. A. Funnel containing turbulent water. B. Funnel containing stationary water. C. Perforated plug of aquarium cement. D. Solid plug of aquarium cement. E. Flask containing water to filter compressed air. F. Compressed air outlet. G. Glass funnel covers. H. Ring stand.

For these preliminary experiments, accurate count of nauplii was not attempted, but nauplii from the egg sacs of each female parent were roughly divided between experimental chambers and control chambers. Some of the parents (both male and female) had the minimum spine formula and some the maximum. Possible influence of heredity is considered later.

Temperature of water in the containers varied from 25° to 27° C. Food (previously described) was added to each container every other day.

Results of Experiment I. (see Table 2): In each vessel, nauplii took about 14 days to mature fully. Twenty-one adult copepods were recovered from turbulence chambers and 30 from controls.

There was no measurable difference in body length between "activated" animals and controls; neither was there a difference in proportions of parts of the body (swimming feet and furcal rami); but there was a difference in the number of spines on the terminal segments of the exopods of the four pair of swimming feet.

Copepods in the turbulence chambers gave a higher percent of increase in number of spines over the minimum than did those of the controls (percentages secured using formula previously given). Twenty-one turbulence copepods showed an 83.3% increase, but 30 controls only a 28.3% increase.

For an additional experiment with air-caused turbulence, during October and November 1947, 104 nauplii were evenly divided between turbulence and control vessels which varied in temperature from 19° to 24° C.

Results of Experiment II. (see Table 2): Survival rate was very low; only eight adults were recovered from the turbulence vessel and seven from the control vessel. The former showed a 53.0% and the latter a 55.0% increase over the minimum.

(b) Stirrer-caused Turbulence Experiments

Material and Methods (Experiments III & IV): To eliminate the possible factor of extra oxygen in the air bubble-caused turbulence chambers, stirrers were secured. J. L. Brooks (1947) had found electric stirrer-caused turbulence to be effective in producing morphological changes in certain species of Daphnia. When using stirrers

TABLE 2. - COMPARISONS OF COPEPODS REARED IN TURBULENT AND NON-TURBULENT WATER

EXPERIMENT NO.	DATE OF EXPERIMENT	TEMPERATURE (CENTIGRADE)	SPINE FORMULA OF PARENTS	NO. OF ♂♂ & ♀ NAUPLII PUT IN	NO. OF ♂♂ & ♀ COPEPODS RECOVERED	% INCREASE IN NO. SPINES IN ♂♂ & ♀♀	NO. OF ♂ COPEPODS RECOVERED	% INCREASE IN NO. SPINES IN ♂♂	NO. OF ♀ COPEPODS RECOVERED	% INCREASE IN NO. SPINES IN ♀♀
I	APR. & JUNE 1947									
	*TURBULENCE →	25°-27°	{2,3,3,3 & 3,4,4,4	OVER 50	21	83.3%	2	100.%	19	80.1%
	CONTROL →	25°-27°		OVER 60	30	38.3%	16	48.4%	14	26.8%
II	OCT. & NOV. 1947									
	*TURBULENCE →	19°-24°	2,3,3,3	52	8	53.%	5	62.5%	3	37.5%
	CONTROL →	19°-24°	2,3,3,3	52	7	55.%	4	47.%	3	66.6%
III	DEC. 1947									
	TURBULENCE →	17.3°-21.6°	2,3,3,3	30	10	83.7%	5	75%	5	92.5%
	CONTROL →	17.3°-21.6°	2,3,3,3	29	15	73.3%	7	59%	8	86.0%
IV	DEC. 1947									
	TURBULENCE →	19.°-22.5°	2,3,3,3	47	10	65.%	3	96%	7	51.8%
	CONTROL →	19.°-22.5°	2,3,3,3	46	10	47.5%	1	100%	9	41.7%
V	FEB. 1948									
	TURBULENCE →	26.2°-26.5°	2,3,3,3	115	105	55.%	44	34.3%	61	70.%
	CONTROL →	26.2°-26.5°	2,3,3,3	115	104	39.4%	55	25.%	49	55.6%
VI	MAR. 1948									
	TURBULENCE →	28.1°-28.5°	2,3,3,3	81	26	43.7%	5	20.%	21	49.4%
	CONTROL →	28.1°-28.5°	2,3,3,3	79	39	37.1%	8	23.4%	31	40.3%
VII	SEPT. 1949									
	TURBULENCE →	29.45°-29.5°	2,3,3,3	99	26	55.7%	11	44.3%	15	64.16%
	CONTROL →	29.45°-29.5°	2,3,3,3	98	43	43.0%	20	38.1%	23	47.3%
VIII	OCT. 1949									
	TURBULENCE →	29.4°-29.5°	2,3,3,3	154	84	69.9%	30	60.%	54	75.4%
	CONTROL →	29.4-29.5°	2,3,3,3	156	107	37.3%	41	35.7%	66	38.4%

*-TURBULENCE CAUSED BY AIR BUBBLES; STIRRERS USED IN ALL OTHER EXPERIMENTS.

I found by use of the Winkler method, as had Brooks, that the oxygen concentration was practically the same in turbulence as in the control vessels. Because electric stirrers often become hot and cause an undesirable rise of water temperature, the stirrers used in my experiments were made to revolve by compressed air. They never heated even after long running. The stirring unit was a simple glass rod having a slight curve or kink near the lower end (fig. 10). It revolved at a moderate rate, or just fast enough to keep the debris in the vessel moving around in the current.

Containers for both experimental and control animals were cylindrical, flat-bottomed, museum jars of 800 cc. capacity, 4 inches in diameter and 5 inches high.

No attempt was made to regulate temperatures in the experiments of December, 1947, but frequent checks were made to determine the temperature ranges of all vessels. These are shown in Table 2, (Experiment III & IV). Low temperatures were not employed because low temperature alone would permit most of the animals to have the high spine formulas and thus mask the effects of turbulence.

To prevent air of the compressed-air stirrer from blowing on the water of the turbulence chambers and thus causing too rapid evaporation of water and lowering of water temperatures, a cellophane cover, with a hole for the stirrer, about the diameter of a 25 cent piece, was placed over the top of each turbulence chamber. Similar covers with holes were placed over control vessels.

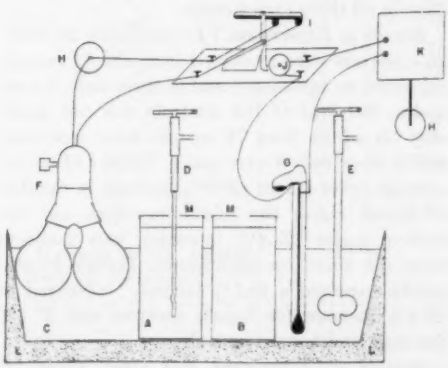

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FIG. 10. Stirrer-caused turbulence apparatus. A. Museum jar containing turbulent water. B. Museum jar containing stationary water. C. Dish-pan water bath. D. Compressed-air stirrer to create turbulence. E. Compressed-air stirrer to circulate water bath. F. 20 watt electric light bulbs. G. Thermoregulator. H. A.C. outlet. I. Mercury column. J. Electro magnet. K. Transformer. L. Rock wool insulation. M. Celophane museum jar covers.

As before, nauplii from each pair of egg sacs were divided between turbulence vessel and control vessel. In these and the following experiments accurate counts were made of nauplii, which were divided approximately equally. Both parents of the nauplii of these and the later experiments had the minimum spine formula.

Results of Experiments III and IV. (see Table 2.): Survival rate was low, only one male control being recovered from Experiment IV. Nevertheless, when males and females were considered together, increase in number of spines above the minimum was 83.7% for turbulence-treated to 73.3% for controls in Experiment III and 65.0% for turbulence-treated to 47.5% for controls in Experiment IV.

(c) *High Temperature-Turbulence Experiments*

Material and Methods (Experiments V, VI, VII, & VIII): To regulate temperature accurately and to secure higher temperatures in the vessels, a water bath was improvised (see fig. 10).

A large dishpan (L), around which was packed rock wool for insulation, was used as a container to hold both control and turbulence vessels. Water in the pan was kept circulating with a compressed-air stirrer (E). Heat was provided by two 20 watt electric light bulbs which extended about half their lengths into the water of the pan. Two bulbs were used as a precaution, since one bulb would maintain the desired temperature of the water if the other burned out. The source of heat was equidistant from the turbulence and control chambers, although not appearing so in the two-dimensional sketch (fig. 10).

Except for some details of dimensions the plan of the apparatus for regulating temperature was essentially the same as that of one of the small chambers of the machine described by Coker and Constable (1936). The 110 volt circuit for the light bulbs was controlled by a 6.3 volt relay. This low voltage current was derived from the regular electric circuit through a small transformer. A rectifier to convert A.C. to D.C. would be useful, but is not essential since only a "buzzing" of short duration is caused by the A.C.

When the column of mercury of a thermoreg-

ulator, partly immersed in the bath, rises to the level of the upper electrode, the low voltage relay is closed, thus bringing into operation an electromagnet which pulls a switch that tilts an ordinarily level mercury column and breaks the 110 volt circuit that supplies the heat. When the column of mercury of the thermoregulator falls, the low voltage circuit is broken, the magnet ceases to operate and the tilted column of mercury becomes level again to complete the 110 volt circuit and turn on the heat. So effective is this control that the temperature of turbulence and control chambers ordinarily varies only within limits of less than 0.4° C.

Evaporation from the water in the bath was appreciable, and was compensated for by adding each day about 1000 c.c. of water raised to the temperature of the bath.

This apparatus, without a refrigerating unit, is effective only in experiments in which the temperature is kept above that of the surrounding air; the entire apparatus can be easily moved, however, and since low speed electric motors can be used to replace the compressed air stirrers, it can be set up anywhere there is an electric circuit, as in a cold room. Use in an open room as in these experiments has the advantage of giving turbulence and control chambers sufficient daylight to promote increase in the supply of food for the copepods.

Nauplii of *C. carolinianus* parents with the minimum spine formula were divided approximately equally between turbulence chamber and control chamber. Although the genetics of the parents was unknown, the random assortment of nauplii from a single pair would seem to preclude a differential genetic effect in turbulence and control chambers.

Temperature of the chambers in the February, 1948, experiment (No. V) varied from 26.2° to 26.5° C. At this higher temperature the copepods matured in about 7 days.

Results of Experiment V. (see Table 2): From 115 nauplii subjected to turbulence 105 adult copepods were recovered and these showed a 55% increase in number of spines over the minimum. From 115 control nauplii 104 adults were recovered and these showed a 39.4% increase in number of spines over the minimum (see Table 2. Experiment V). When the sexes are treated separately, we find an increase of 34.3% in 44 experimental males, and 70.0% in 61 experimental females, compared to 25% in 55 control males, and 55.6% in 49 control females (see Table 2). There is some evidence that females tend to have more spines than do males reared at the same temperature.

There was no measurable difference in body length nor in proportion of parts between experimental animals and controls.

Before the results of Experiment V were available, there was thought of a possibility that the small but usually constant difference in spine number between experimental animals and controls might be the result of differential survival. In other words, most of the animals able to survive in turbulent water could be those that had more spines, which enabled them to swim more effectively or to cling more efficiently to the sides and bottom of the container. The spination patterns developed in this experiment along with a notable high rate of survival reduces this possibility.

In Experiment VI (March, 1948), temperature of the containers was kept between 28.1° and 28.5° C.; in Experiments VII and VIII (September and October 1949), temperatures varied between 29.4° and 29.5° C. These high temperatures were used in an attempt to make the controls approach more closely the minimum spine formula. The animals matured in about 6 days in all three experiments.

Results of Experiment VI. (see Table 2): Only 26 copepods were recovered from the 81 nauplii subjected to turbulence and of these only 5 were males. Survival of the controls was not good, only 39 adults from 79 nauplii were recovered and of these only 8 were males. Males had on the average fewer spines (20.0% increase in number of spines above the minimum) than did the control males (23.4% increase), but numbers were too small for significance. Female experimentals showed a 49.7% increase, compared to 40.3% increase for female controls and 37.1% for male and female controls.

Results of Experiment VII. (see Table 2): Survival of copepods in this experiment was not good, but more males were recovered than in Experiment VI. Increase in number of spines was as follows: 11 male experimentals—44.3%; 20 male controls—38.1%; 15 female experimentals—64.16%; 23 female controls—47.3%; male and female experimentals—55.7%; and male and female controls—43.0%.

Results of Experiment VIII. (see Table 2): Survival rate was good. Increase in number of

spines was as follows: 30 male experimentals—60%; 41 male controls—35.7%; 54 female experimentals—75.4%; 66 female controls—38.4%; male and female experimentals—69.9%; and male and female controls—37.3%.

During these experiments (VI, VII, VIII), the copepods could be observed after they achieved the early copepodid stages. Those in the turbulent water tended to cling to the sides of the container and made short forays only to secure food or when apparently swept off by the water current. Those in the control vessel spent about as much time swimming and crawling as they did remaining attached to the side of the container. Almost invariably the control females were observed with egg sacs several days before such were seen on the experimental females. It may be that the experimental animals develop slightly more slowly; or the current may hamper the male in capture of the female.

(d) Aggregation of Turbulence Experiments

In Table 3, numbers of copepods and total spine counts for each experiment are aggregated. Females from turbulent water had a 69.3% increase above the minimum number of spines as compared with 45.5% for the control females. Turbulence males had a 48.3% increase in spines as compared to 34.52% for the control males. Lumping males and females together, turbulence copepods have a 61.76% spine increase as compared to 40.84% for the controls.

DISTRIBUTION OF SPINE NUMBERS IN EXPERIMENTAL AND CONTROL ANIMALS

Figures number 11 through 16 show how spine numbers are distributed in both controls and experimental animals. Among the controls, not considering sex (fig. 11, left), the greatest number of animals had the minimum spine formula

TABLE 3

Turbulence

Experiment	Females		Males		Males & Females	
	No. copepods	No. spines	No. copepods	No. spines	No. copepods	No. spines
I	19	542	2	60	21	602
II	3	75	5	135	8	210
III	5	147	5	140	10	287
IV	7	183	3	89	10	272
V	61	1684	44	1089	105	2773
VI	21	545	5	118	26	663
VII	15	407	11	281	26	688
VIII	54	1514	30	804	84	2318
	185	5094	105	2716	209	7813
% Increase in No. Spines Over the Minimum	69.3%		48.3%		61.76%	
			Control			
I	14	338	16	414	30	752
II	3	282	4	103	7	185
III	8	231	7	187	15	418
IV	9	228	1	30	10	258
V	49	1296	55	1320	104	2616
VI	31	782	8	191	39	973
VII	23	593	20	501	43	1094
VIII	66	1655	41	1019	107	2674
	203	5205	152	3765	355	8970
% Increase in No. Spines Over the Minimum	45.504%		34.62%		40.84%	

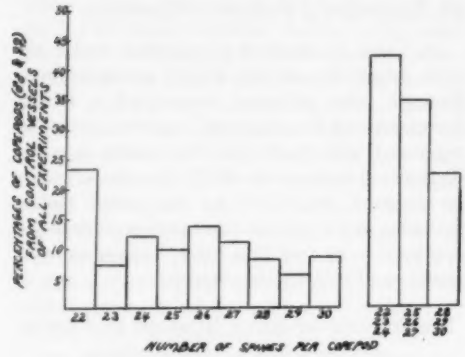

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FIG. 11. Percentages of copepods (♂♂ & ♀♀) from control vessels of all experiments.

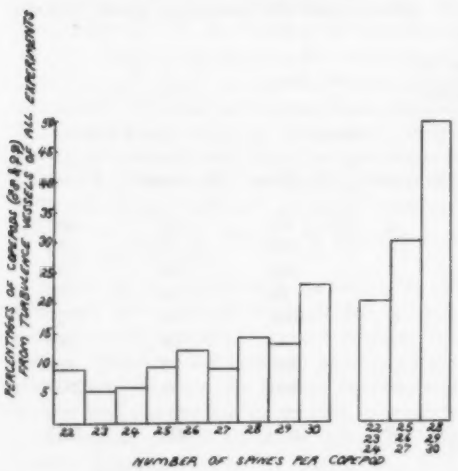

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FIG. 12. Percentages of copepods (♂♂ & ♀♀) from turbulence vessels of all experiments.

(22 spines). By lumping together, respectively, copepods with 22, 23, 24, those with 25, 26 and 27, and those with 28, 29 and 30 (fig. 11, right); it is found that the greatest number of individuals are in the lowermost spine count bracket, fewest in the high bracket, and an intermediate number in the middle bracket.

Among the turbulence animals, disregarding sex, the greatest number of the copepods had the maximum spine formula (30 spines) (fig. 12, left); by lumping, as for the controls, a graph (fig. 12, right) is secured which is the reverse of the control graph.

When the sexes are considered (fig. 13, 14, 15, 16), it is evident that most female control copepods (fig. 13, left) gave intermediate spine numbers and most female experimental copepods have the high spine number (fig. 14, left). Although the greatest number of male copepods in both controls (fig. 15, left) and experimentals (fig. 16, left) have the minimum spine number, yet, when we lump the spines as above, a majority of male controls have the lower spine counts,

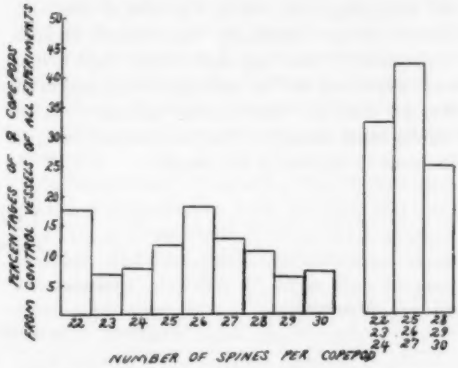

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FIG. 13. Percentages of ♀ copepods from control vessels of all experiments.

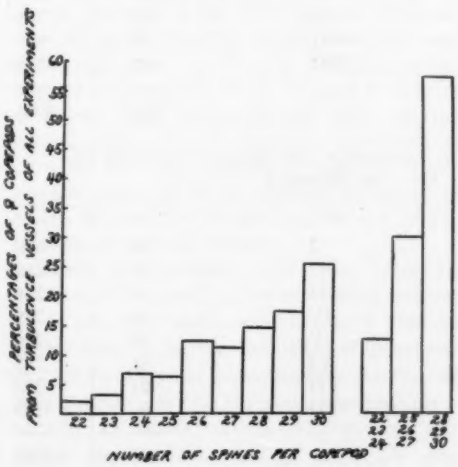

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FIG. 14. Percentages of ♀ copepods from turbulence vessels of all experiments.

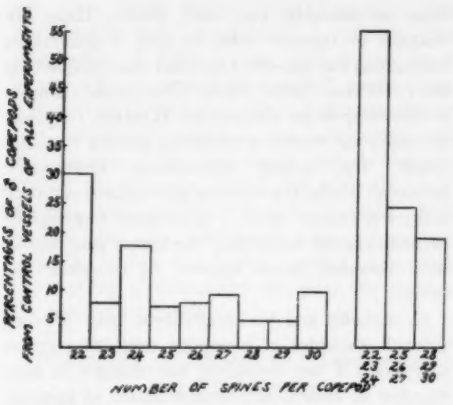

FIG. 15. Percentages of ♂ copepods from control vessels of all experiments.

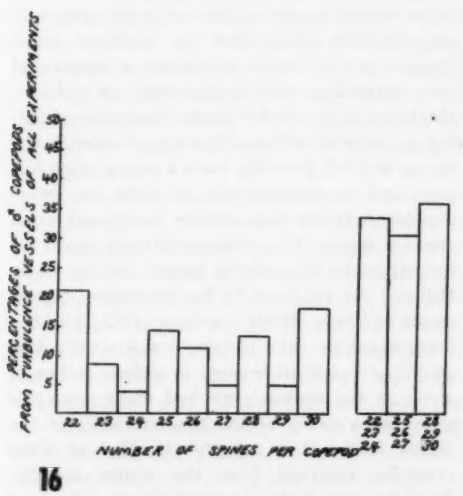

FIG. 16. Percentages of ♂ copepods from turbulence vessels of all experiments.

while a greater number of male experimentals are in the high spine count class than are found in either the low or intermediate spine count classes.

STATISTICAL SIGNIFICANCE TEST

In order to test the significance of the results, analysis was made using large-sample theory, including central limit theorem. I wish to thank Dr. Robert Hooke, formerly of The University of the South faculty, for suggesting this test and helping with it. Because this test may be of

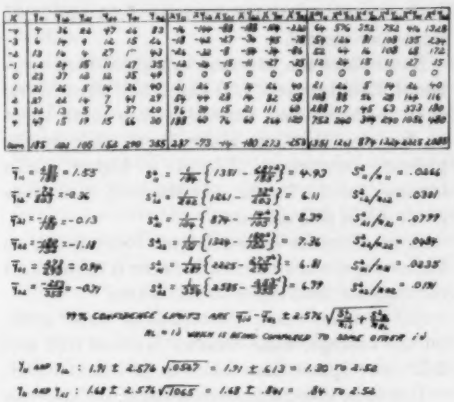

FIG. 17. Mathematics of statistical test.

considerable interest to biologists, its mathematics are given completely in figure 17. In this figure the following symbols are used:

x = number of spines per animal, minus 26

$y_{ij} = f_{ij}(x)$ = frequency of animals having x spines where

$i = 1, 2, 0$, according as we consider females, males, and both together

$j = 1, 2$ according as we consider experimental or control animals

s_{ij} = standard deviation of x for the distribution f_{ij}

$\bar{y}_{ij}$ = mean of x for the distribution f_{ij}

n_{ij} = number of animals counted in the distribution f_{ij}

Although the population is not normal, the samples are large enough (all over 100) to justify assuming that the sample means are normally distributed. On this assumption, using 99% confidence limits, we interpret the results as follows:

1. The effect of turbulence on all animals (both males and females) is, on the average, to add some number between 1.12 and 2.18 spines per animal.

2. For females, the effect of growth under turbulent conditions as revealed in our experiments was to add, on the average, some number between 1.30 and 2.52 spines per animal.

3. For males the effect was to add, on the

average, some number between 0.13 and 1.97 spines per animal.

Note: Since the two intervals given here overlap (1.97 is between 1.30 and 2.52), there is no valid evidence that turbulence has more effect on females than on males, even though the present experiment showed a higher mean increase for the former. (At the 95% confidence level, this is still the case.)

4. (a) Females reared in still water grow, on the average, some number between 0.10 and 1.54 more spines than males in still water.

(b) Females reared in turbulent water grow, on the average, some number between 0.84 and 2.52 more spines than males in turbulent water.

It is to be noted that each of the above intervals fails to include zero, so that there is a significant indication of actual effect (at the 99% confidence level) in every case.

Using only data from Experiments V, VI, VII, and VIII (experiments in which temperature of both turbulence vessels and controls was accurately regulated and survival rates were generally good) and applying the same statistical test at the 99% confidence level, we interpret the results as follows:

1. The effect of turbulence on all animals is, on the average, to add some number between 1.04 and 2.15 spines per animal.

2. The effect of turbulence on females is to add, on the average, some number between 1.38 and 1.97 spines per animal.

3. The effect of turbulence on males is to add, on the average, some number between 0.76 and 1.85 spines per animal.

4. (a) Females reared in still water grow, on the average, some number between 0.39 and 1.80 more spines than males in still water.

(b) Females reared in turbulent water grow, on the average some number between 1.2 and 2.93 more spines than males in turbulent water.

These results correspond very well with those for all the experiments combined.

Comparison of Cyclomorphosis in Daphnia and in Certain Cyclopoid Copepods

Cyclomorphosis refers to the cycle of different forms encountered throughout the year in certain species of rotifers, dinoflagellates, cladocerans, etc. There is a sequence of generations each characterized by progressive changes in appearance resulting in a generation noticeably different from an ancestral one; and, finally, there are changes in reverse order to give a generation resembling the ancestral one and thus completing the cycle (see Coker, 1939). The classic example is the European cladoceran *Daphnia cucullata* in which the winter generations exhibit rounded heads, the spring generations moderately helmeted heads, the summer generations strongly helmeted heads, the fall generation moderately helmeted heads, and finally the winter generations with rounded heads appear, to complete the cycle.

C. vernalis and *C. carolinianus* may be considered examples of copepods exhibiting cyclomorphosis if one considers the changes in size, number of spines, and slenderization of appendages and caudal rami. Examples that mature in cold winter water generally are relatively large, usually have the maximum spine formula, and slender caudal rami and appendages, whereas those reared in warm summer water generally are relatively small, have the minimum spine formula or a reduction in number of spines and have appendages and caudal rami not quite so slender as in the winter forms. Examples maturing in water of intermediate temperature in the spring and fall generally have a mixed spine formula and, in slenderization of parts, are intermediate between large winter forms and small summer forms. These statements are based on collections by the present author and by L. L. Hill and are bolstered by the laboratory experiments of Coker (1934a), Aycock (1942), and the present author. The picture is not always this simple and clearcut because in certain bodies of water in the summer, we find many examples which show many of the characteristics of the winter forms. It is not probable that all these examples survived from the winter months. Possibly such factors as turbulence influenced some morphological changes (number of spines), though there may be other factors.

In a collection made by Dr. R. E. Coker from Lake Chautauqua, New York, August 25, 1928, the *brevispinosus* form of *C. vernalis* with the maximum spine formula accompanies *Daphnia retrocurva*, which exhibits large curved helmets. Higher temperature plus turbulence and perhaps some other factors may have permitted the production of helmets on *Daphnia retrocurva* (see Coker and Addlestone, 1938; Coker, 1939; Brooks, 1947), whereas, turbulence and possible other factors could have produced the *brevispino-*

sus form (with higher spine formula); high temperature alone would tend to influence the production of fewer spines. This would be in accord with our experiments with *C. carolinianus*.

In certain species of Daphnia, high temperature and turbulence influence the production and maintenance of the same result, namely helmets, spikes, etc. (Brooks, 1947); but with *C. carolinianus*, and possibly with *C. vernalis*, high temperature and turbulence seem to produce opposing effects, the former tending to decrease the number of spines and the latter to increase the number of spines.

DISCUSSION OF EFFECTS OF TEMPERATURE AND TURBULENCE

The influences of temperature and turbulence seem to be exercised during all nauplial and early copepodid stages, the effects possibly being cumulative during these stages of development. Some preliminary "high" temperature experiments using a very few nauplii in warm water (transfer made during late nauplial stages) resulted in more adult copepods with intermediate spine numbers (instead of low spine numbers) than if the transfer of nauplii had been made at earlier stages.

Presumably both temperature and turbulence act through an influence on the metabolism of developmental stages. High temperatures without turbulence tend to increase metabolic rate; development is speeded up; and small copepods with fewer spines are produced. Conversely, low temperatures influence the development of large copepods with more spines, and high temperatures plus turbulence influence the development of small copepods with more spines. It is questionable whether turbulence increases the metabolic rate of *Cyclops carolinianus*. As noted previously, this species, unlike many cladocera and many other copepods, is not a water-treader and as noted above, copepodid stages observed in the turbulent water tended to cling to the sides of the container and made short forays out into the water. Actually, turbulent water often brings food within reach of the stationary copepod. Copepods observed in the quiet control vessel seemed to swim considerably more than those in the turbulence chamber; they have egg sacs sooner; presumably they have a higher metabolic rate. This might explain why high temperatures and turbulence produce similar effects in certain species of water-treading Daphnia and opposing effects in *Cyclops carolinianus*.

The possibility of a genetic explanation of the results should not be overlooked. Some species of Daphnia and Cyclops exhibit pronounced cyclomorphosis in slight degree or not at all. Penetrance and expressivity may be associated with this phenomenon. The term penetrance is used to indicate the regularity with which a gene produces its effect. A dominant gene has complete penetrance if it produces its effect in all individuals that carry it, a recessive gene has complete penetrance if it produces its effect in all individuals which are homozygous for it. If some individuals fail to show phenotypically their genotypic potentialities, the gene is said to have reduced penetrance. Environmental conditions (temperature, culture conditions, etc.) may inhibit penetrance, as is shown in the case of lobe eye in Drosophila. Expressivity is a measure of the kind or amount of effect shown in an individual possessing the gene. The recessive gene for vestigial wings in Drosophila seems to have complete penetrance (in the homozygous condition), but varies in expressivity. Flies reared at 70° F. have tiny wings; those reared at 88° F. have wings almost normal in length.

In some cases, penetrance varies with the environment, while expressivity is nearly uniform. Insufficiency of food for Drosophila with the genotype for "giant" results in fewer giants, but all giants are nearly the same size. In other cases penetrance and expressivity are both involved in the results. I am unable to say whether penetrance or expressivity, or both or neither, are involved in the results obtained with temperature and turbulence. Extensive mating experiments will have to be done to determine the role of genetics in cyclomorphosis.

Attempts have been made to determine if copepods with high spine count have any advantages over those with low spine count. Perhaps the spines aid in clinging to objects or in swimming in turbulent water. High rate of survival of copepods in Experiment V tends to eliminate the possibility that spine development is quite independent of turbulence but that survival in turbulent water is for those that by chance have the maximum spine number. The results in some other experiments, notably No. VIII, seem corroborative. In the natural environment, survival rates of such small animals cannot well be checked. There is no need to assume that every

character or modification of character is of advantage to the organism.

SUMMARY

When there is a fair percentage of survival, low temperature and turbulence, either alone or together, tend to permit the development of animals showing more spines than those reared at high temperatures or in non-turbulent water. This may be expressed in tabular form:

	Low temperature	High temperature	High temperature and turbulent water	High temperature and non-turbulent water
% increase in no. of spines over minimum	High %	Low %	Moderately high %	Low %

Low temperature alone is more effective in increasing the number of spines than is turbulence (see Tables 1 & 3). Considering only the number of spines produced, it masks the effects of turbulence, because both are followed by the same effect in spine development. The few copepods reared at low temperature in turbulent water could not be distinguished in respect to spine count from those reared at low temperatures alone.

High temperature does not nullify the effect of turbulence in respect to spine development: the spine counts of animals reared at high temperatures are high under conditions of turbulence, low with non-turbulence.

From the evidence of these experiments, body length and proportions of segments of swimming feet and furcal rami are definitely influenced by temperature prevailing during the period of development but are not affected by turbulence or non-turbulence.

Seasonal changes in form, often exhibited by *C. vernalis* and *C. carolinianus* with respect to size, number of spines, and slenderization of parts of the body may be considered cyclomorphic changes comparable in some respects to the cyclomorphic changes found in certain species of Daphnia. In the case of Daphnia, high temperature and turbulence, and possibly other factors, cause production of helmets, spikes, etc.; with *C. carolinianus*, and possibly with *C. vernalis*, high temperature and turbulence seem to have opposite effects in respect to influence on production of spines. This could possibly account for the fact that copepods found in warm water during the summer months may exhibit certain characteristics of winter form (increase in spine number).

There is some evidence that the influences of temperature and turbulence seem to be exercised during nauplial and early copepodid stages; the effects are possibly cumulative during these stages.

Presumably both temperature and turbulence act through an influence on the metabolic rate of the animals. High temperature speeds up development and produces smaller, fewer-spined animals. Turbulence may increase the activity and speed up the metabolic rate of water-treaders, such as *Daphnia ambigua*, but cause *Cyclops carolinianus* to become less active as it clings to sides of the container. This may account for the similar effects of turbulence and high temperatures in a water-treading species such as *Daphnia ambigua* and opposing effects in *Cyclops carolinianus*.

Since species closely related to and living with those exhibiting cyclomorphosis fail to show different seasonal forms, genetics must play an important role in cyclomorphosis. Environmental conditions may inhibit penetrance or expressivity in certain species of Daphnia and Cyclops, but extensive mating experiments are needed to determine if penetrance or expressivity are involved in the results obtained by rearing copepods at different temperatures and in turbulent and non-turbulent water.

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BORING SPONGES (CLIONIDAE) OF NEWPORT RIVER, NORTH CAROLINA

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Although Old (1941) made a collection of boring sponges in Beaufort Harbor, no extensive study has been made of their distribution in the brackish waters of North Carolina. The pattern of distribution of the several species is of particular interest for comparison with recent studies in Louisiana and South Carolina (Hopkins, 1956a, 1956b). Although boring sponges in Louisiana exhibited a zonation that correlated with zones of brackish water, no such pattern was discernible in South Carolina waters.

Because boring sponges attack the shells of living oysters they are a pest to commercial oystermen. Their penetration weakens the shells of oysters sufficiently to make them less desirable for the market. By stimulating the production of extra layers of shell, boring sponges divert the energy of oysters that otherwise might be utilized in the production of edible meats. In addition, Lunz (1943) has suggested that the high incidence of *Cliona* in waters of high salinity prevents the development of oysters below the low tide mark. Information on the distribution of these boring sponges should aid in the choice of locations suitable for the cultivation or "farming" of oysters.

This paper reports the distribution of boring sponges in Newport River, North Carolina, over an eighteen-month period, including a period of extremely low salinities caused by several hurricanes. This report is based on a broad study of the fauna of oyster beds and their distribution in relation to salinity, submitted to Duke University as a dissertation in partial fulfillment of the requirements for the Ph.D. degree. The author wishes to express his appreciation to Dr. I. E. Gray, under whom this study was made, and to Dr. C. G. Bookhout, who made the facilities of the Duke Marine Laboratory available to the author.

Methods

Living oysters and shell were collected at monthly intervals from May, 1955, to October, 1956. Collections were limited to a zone between the high tide mark and the level approximately two feet below normal low tide. No effort was made to collect every boring sponge present; instead, the collections were designed to sample many types of oyster associates and, therefore, should provide a reliable estimate of sponge infestation.

All shells were examined for the presence of boring sponge colonies. Usually, colonies of *Cliona celata* can be distinguished from other species by the larger size of the shell perforations (2 to 3 mm *vs.* 0.5 mm in the other species). For identification of other species, spicule preparations are needed for microscopic examination. A small piece of sponge tissue from each colony was placed on a microscope slide, to which were added several drops of sodium hypochlorite (Clorox). Often it was necessary to remove shell fragments to obtain sponge tissue from underlying galleries in the shell. When the preparation ceased foaming, the remaining sponge spicules were examined microscopically and compared with descriptions and figures in Old's paper on *Cliona* (Old, 1941).

Oysters less than 30 mm long are not included in the enumerations, for such young oysters were rarely infested and the inclusion of great numbers of newly set spat would give a misleading picture of *Cliona* infestation for the population of marketable oysters.

Collecting Stations

Newport River empties into Beaufort Inlet between Beaufort and Morehead City, N. C. (Fig. 1). Although Marshall (1951) regarded Newport River as simply an arm of the sounds, it exhibits a salinity gradient as a result of seepage and runoff from land, and of flow from a non-tidal portion lying in the Coastal Plain north and west of Beaufort Inlet. Core Creek Canal of the Intracoastal Waterway and several drainage canals provide channels for a limited exchange of water with Pamlico Sound to the north. The tidal portion of Newport River extends five miles west of Core Creek, where a bottom of soft mud predominates, interspersed by natural oyster reefs. A number of private oyster beds are located in this area. Below Core Creek, low islands of dredge waste and salt marsh occur in the river.

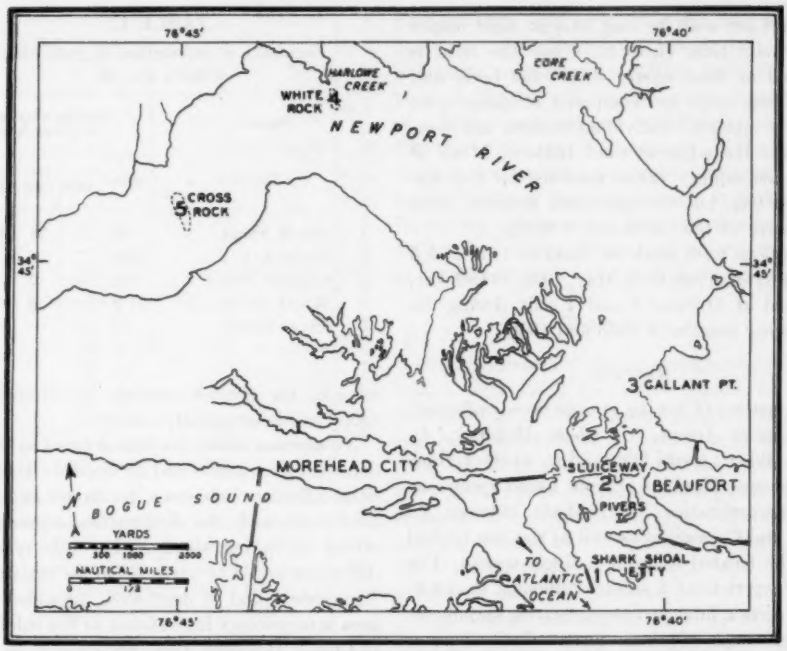

FIG. 1. Chart of Newport River showing location of collecting stations.

Two deep channels lead to the inlet and Bogue Sound through this lower portion, where sand bottoms predominate.

Each collecting station is described below and indicated in Figure 1.

1. Shark Shoal.—Collecting was done on a rock breakwater 550 yards long that parallels Beaufort Channel. Here oysters (*Crassostrea virginica*) are chiefly intertidal, with only individuals scattered below the low tide mark. However, horse oysters (*Ostrea equestris*) were found below low tide.

This station is exposed to the highest salinities, being situated close enough to Beaufort Inlet to receive wave action from the nearby ocean. Mean salinity for nineteen observations made at monthly intervals is 31.8‰. The lowest salinity recorded is 15.3‰, after a period of unusually heavy and prolonged rain associated with the passage of two hurricanes.

2. Sluiceway.—Collections were made at the entrance to a semi-enclosed tidal lagoon near the Piver's Island road. This entrance is a ten-foot break in a series of rocks and pilings which form the crest of a bar that encloses the lagoon. Oysters are massed on the rocks on both sides of the entrance and are scattered over the bottom. The oyster-covered area is 10 yards wide at its widest point, and less than 50 yards long. De Laubenfels (1947) found this location very suitable for collecting a variety of sponges. The mean of twenty salinity observations in this vicinity is 30.9‰, with a minimum of 10.2‰.

3. Gallant Point.—Extending southward 200 yards from Gallant Point itself is a spit or bar of oyster shell that nearly encloses a mud flat containing many large clusters of living oysters. Collections were made from these oysters, and from scattered individuals and shells at the outer edge of this bar, toward deep water. Of twenty salinity observations at this location, the mean is 28.2 ‰, and the minimum, 7.3 ‰.

4. White Rock.—Just west of the point where Harlowe Creek flows into Newport River lies a broad shoal 220 yards long, composed of oysters, dead shell, and sand. In this part of Newport River, most of the bottom is soft mud. Mean of twenty-one salinity observations at this station is 24.1‰; minimum salinity recorded, after the passage of two hurricanes, is 1.7‰.

5. Cross Rock.—This is a typical oyster reef or "rock," 280 yards long and 10 to 12 yards

wide, that lies with its long axis at right angles to the main tidal currents. While the crest is composed of dead oyster shells, the body and sides of the ridge are composed of many clustered live oysters. Individual oysters are scattered over the adjacent mud bottom. Mean of twenty-one salinity values recorded for this station is 19.0‰, but the minimum recorded after the passage of the hurricanes is 0.6‰.

Collections were made at Stations 1, 2, and 5 at monthly intervals from May, 1955, to October, 1956, and at Stations 3 and 4 only during the the summer months of 1955 and 1956.

RESULTS

Five species of boring sponge were collected: *Cliona celata* Grant, *C. lobata* Hancock, *C. spirilla* Old, *C. truitti* Old, and *C. vastifica* Hancock. Because Hopkins (1956b) found specimens with characteristics intermediate between *C. spirilla* and *C. vastifica* as well as the two typical forms, he treated them as a single species. The author experienced a similar difficulty in determining certain intermediate-appearing specimens. However, most of these intermediates bore a close enough resemblance to the description of *C. spirilla* (Old, 1941) to be placed with typical specimens of that form. Because the distribution patterns of the two forms are different, they are treated as separate species.

A total of 2,706 shells was examined for *Cliona* infestations. In these shells, 282 colonies of boring sponge were found. The number of colonies and shells examined for each station is presented in Table I. Sponge colonies were most common at Shark Shoal and Sluiceway, much less common at Gallant Point and White Rock, and very rare at Cross Rock. The relative infestation of shells at each of these stations closely parallels salinity records, the greater number of colonies being found in higher salinity waters.

TABLE I

Number of shells examined and incidence of Cliona *infestation*

Station		Shells examined			Number of *Cliona* colonies
No.	Location	Live oysters	Dead shell	Total	
1	Shark Shoal	299	429	728	113
2	Sluiceway	364	314	678	134
3	Gallant Point	165	199	364	19
4	White Rock	55	99	154	14
5	Cross Rock	320	470	790	2

TABLE II

Incidence of infestation by individual Cliona *species*

Station		Number of colonies of *Cliona* found				
No.	Location	*celata*	*lobata*	*spirilla*	*truitti*	*vastifica*
1	Shark Shoal	63	—	23	19	8
2	Sluiceway	106	7	9	9	3
3	Gallant Point	13	3	2	—	—
4	White Rock	9	—	5	—	—
5	Cross Rock	2	—	—	—	—

No obvious difference was noticed in the incidence for live oysters and for dead shells. There is little difference between a live oyster and a single dead valve as to the shell surface exposed to the attack of boring sponges. For this reason, no difference would be expected in the infestation of live oysters and of dead shell, provided surface area is of primary importance to the colonization of *Cliona*. However, there appeared to be a more obvious infestation of the shells of horse oysters (*Ostrea equestris*) than of the common edible oyster (*Crassostrea virginica*). Commonly, the shell of a horse oyster was extensively eroded while the edible oyster to which it was attached had a much less extensive infestation.

In Table II the infestations at these five stations are analyzed for inividual species of *Cliona*. *Cliona celata* was by far the most abundant and widespread species, having been collected at all five stations. *Cliona spirilla* and *C. truitti* were second and third in abundance. *Cliona spirilla* was found at all stations but Cross Rock, while *C. truitti* was found only at the two most seaward stations. *Cliona vastifica* and *C. lobata* were least abundant, and not found at the two stations farthest upstream. In addition, *C. lobata* was not found at Shark Shoal, the most seaward station.

EFFECTS OF SALINITY

During the period of study, the overall salinity regime of Newport River showed broad fluctuations. Salinity observations at Station 4 are plotted graphically in Figure 2 with concurrent temperature and precipitation data.

Salinities were unusually high in the period immediately preceding August, 1955. During

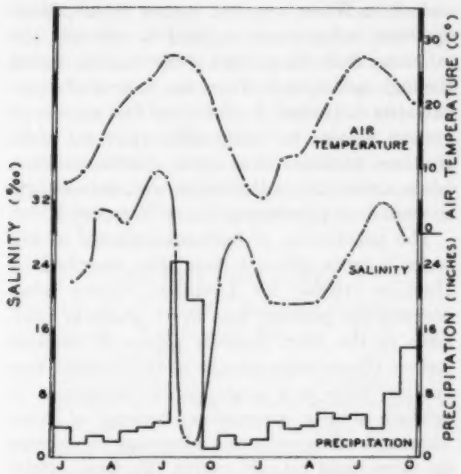

FIG. 2. Precipitation, air temperature, and salinity in upper Newport River, January, 1955, to October, 1956. Precipitation data for New Bern Airport and temperature data for Morehead City from U. S. Weather Bureau (1955, 1956). Letters on abscissa indicate every third month.

this period, *Cliona celata* was collected at White Rock and Cross Rock, *C. spirilla* was collected at White Rock and Gallant Point, and *C. vastifica* was collected at Gallant Point. Apparently this period of high salinity permitted these species to penetrate farther up Newport River. In the period of low salinities that followed, these infestations disappeared. At no other time during this study were these species recorded from these or more landward stations. These stations had not been recolonized by their respective species when this study ended in October, 1956.

August, 1955, was marked by the passage through the area of hurricanes Connie and Diane and by a radical change in the salinity regime of Newport River that resulted from rainfall associated with these storms. Hurricane Ione followed in September and contributed, with other heavy rains, to maintaining the altered salinity regime well into October. Many oyster associates died during this period, and many boring sponge colonies disappeared. Only *Cliona celata*, C. *spirilla*, and *C. truitti* were collected during or immediately after this period at any of the five stations. Only at the two most seaward stations did these species survive, and there they could be found only in reduced numbers.

Salinity conditions returned to "normal" in late October, but early in 1956 low salinities again prevailed. During this period there was no noticeable penetration of *Cliona* into the upper part of Newport River; however, the number of colonies gathered per collection increased at the two most seaward stations. With warmer weather the salinity gradually increased, eventually reaching high values in August, 1956. Increasing numbers of *Cliona* colonies had been collected at Gallant Point, but none were found at the two stations farthest from the ocean.

DISCUSSION

In his collection of boring sponges from Piver's Island, Old (1941) found five species, of which four have been found in this study. Old's fifth species, *Cliona robusta*, is rare. It is known only from the three specimens collected by Old and one collected in South Carolina (Hopkins, 1956b). Although *Cliona truitti* has been collected both north and south of this region (Old, 1941; Hopkins, 1956a, 1956b), it has not been reported previously for North Carolina.

The overall infestation found in this study (10.4%) is low when compared with certain published data. For example, Hartman (1958) found boring sponges in 65 to 80% of the shells that he examined from Long Island Sound, and Hopkins (1956b) found boring sponges in 72% of the shells examined from South Carolina. Since he had set out to demonstrate a distribution pattern for the several species of *Cliona* present, it seems likely that many of the shells examined by Hopkins had been collected because they contained colonies of *Cliona*. For Newport River collections, the inclusion of large numbers of shells from low salinity areas where *Cliona* colonies are rare, has greatly reduced the overall infestation. If this study had been limited to more saline waters, the resulting level of infestation would have been much higher.

The relative abundance of *Cliona celata* in Newport River was similar to that found in South Carolina (Hopkins, 1956b) and Long Island Sound (Hartman, 1958) where *Cliona celata* comprised about two-thirds of the colonies of boring sponges. However, only in the saltiest of six salinity zones did *C. celata* outnumber other species of *Cliona* in the Louisiana study (Hopkins, 1956a); in most oyster beds, where lower salinities prevailed, *Cliona truitti* was the predominant species of boring sponge.

The distribution of boring sponges in Newport

River closely resembled that of boring sponges in South Carolina. Hopkins (1956b) did not find a zonation of species as he had in Louisiana (1956a). He attributed the differences in South Carolina to a recent decline in rainfall and subsequent changes in the salinity regime of South Carolina waters. Increased salinities had permitted a greater penetration of boring sponges into rivers of the area, and he felt that this invasion had obscured any clear distribution patterns. In Newport River, *Cliona celata* was the most abundant species at each station, and nowhere did any of the other species assume a numerical importance rivaling it. *Cliona spirilla*, found to be next in abundance to *C. celata* in Newport River, was treated in the South Carolina study as a form of *C. vastifica*, which species was second in abundance in that study.

While Newport River may have undergone a long-term change in salinity conditions such as that described by Hopkins for South Carolina, obscuring any distribution pattern for *Cliona* species, no available information indicates this. In this connection, some comments in scientific publications involving the salinity regime of Beaufort Harbor are misleading (de Laubenfels, 1947; Hartman, 1958). Most are based on a series of observations made daily over a five-year period by Gutsell (1931), of which only a table of the monthly minimum and maximum salinities for the five-year period is generally available. Two of these years were marked by the occurrence of freshets in early fall similar to the period of low salinity that occurred during this study as a result of rainfall associated with tropical storm systems. Probably these freshets were due to similar tropical storms. De Laubenfels (1947) visited Beaufort in an unusually rainy summer and found salinities as low as 15‰ at Piver's Island. The minimum salinities found by Gutsell and de Laubenfels are rare. Ordinarily, salinity conditions in Beaufort Harbor range between 30 and 35‰. Maturo (1959) never found salinities in Beaufort Harbor below 28‰ during the year preceding this study. The only time during this study when values were recorded at Piver's Island below 25‰ was after the extremely heavy rainfall from the hurricanes—twenty inches in one week (U. S. Weather Bureau, 1955).

When allowed a long enough period of high salinity, as occurred in 1955, colonies of *Cliona* become established at upstream beds of oysters and shell. When a severe freshet occurs, these upstream colonies are exposed to critically low salinities. Both the pattern of distribution during the high salinity period and the pattern of survival after a freshet should show the success of various species in penetrating upstream beds. By these criteria, *Cliona celata*, *spirilla*, *vastifica*, *lobata*, and *truitti*, in that order, were decreasingly successful in penetrating upper Newport River.

The penetration of individual species in this series is quite different from that described by Hopkins (1956a) for Louisiana. *Cliona celata* occupies the position held by *C. truitti* in Louisiana as the most common species in brackish waters. *Cliona celata* may be more successful here because there is a larger parent population to produce a new generation, because of some unrecognized reproductive advantage, or because its more rapid rate of growth (Hartman, 1958) gives it an advantage in competing for substrate. Similar differences, as well as differences in salinity tolerance, may permit different species of boring sponge to flourish in other areas. In this regard, records listed by Old (1941) for *Cliona* species in Chesapeake Bay resemble distribution in Louisiana. These two areas probably possess a more stable salinity regime than that found in Newport River or South Carolina. It seems that a stable salinity regime may favor the development of extensive populations of *Cliona truitti*.

Some Louisiana rivers contain a much higher concentration of calcium than do any corresponding rivers in the North Carolina Coastal Plain (Clarke, 1924). Could a higher concentration of calcium in freshwater sources selectively influence the survival of one species of boring sponge over another in adjacent brackish waters? Calcium is recognized as an important factor in the survival of organisms in low salinity waters (Weil and Pantin, 1931).

Evaluation of these various factors must await laboratory study of *Cliona* species under controlled conditions.

SUMMARY

Distribution of boring sponges in Newport River has been studied over an eighteen-month period. Five species were found. Fewer colonies and fewer species were found on upstream oyster beds. Colonies established on upstream beds under high salinity conditions were killed during extremely low salinities caused by heavy precipi-

tation during passage of three hurricanes. Recolonization was delayed, and incomplete one year later. *Cliona celata* was most abundant and apparently most successful in invading upstream oyster reefs. There was no brackish water zone in which one of the other four species became more abundant, as has been found in Louisiana waters. Instead, distribution of boring sponges in Newport River resembles distribution in South Carolina waters.

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HEMOGLOBIN CONCENTRATION AND OXYGEN CAPACITY OF MAMMALIAN BLOOD[1]

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Introduction

Although there exists a large literature on hemoglobin concentration and oxygen capacity determinations of mammalian blood, values for both are rarely quoted simultaneously. Knowledge of the relation between oxygen capacity and hemoglobin concentration offers certain advantages. For example, hemoglobin values for the blood can be multiplied by a constant to obtain oxygen capacity, or by the reverse procedure, the hemoglobin concentration can be arrived at from the oxygen capacity. Oxygen capacity, the basis for constructing oxygen dissociation curves (100% saturation), when arrived at by two independent means insures more accurate curves, and indirectly indicates whether oxygen content measurements (less than 100% saturation) have been accurately established by the gasometric methods.

The determination of a constant for arriving at oxygen capacity from hemoglobin values of human blood was reported by Morrison (1933). By measuring directly oxygen capacity of hemoglobin, the value 1.3 ml O_2/g. Hb was obtained. Morrison and Hisey (1935) revised the constant for human hemoglobin to 1.34 ml O_2/g. Hb. These authors also established the value 1.34 for beef and 1.355 for dog hemoglobin by use of the CO capacity and iron content of the crystalline samples. The value used at the present time for human hemoglobin is usually 1.36 ml O_2/g. Hb, which was established by Bernhart and Skeegs (1943). Their method was essentially to determine the iron content of hemoglobin, which was found to be 0.34 per cent, and to calculate the constant from this value, by assuming that one mole of iron binds one mole of oxygen. Additional evidence was presented by Jope, Jope and O'Brien (1943) who obtained the value 396 ml O_2/g. Fe for human hemoglobin, which is very close to the theoretical value of 401 ml O_2/g. Fe. Finally, Ramsay (1946) found similar values for sheep and horse hemoglobins.

In the present study, hemoglobin and oxygen capacity determinations were interpreted directly in terms of the volume of oxygen carried per gram of photometrically determined hemoglobin.

Materials and Methods

Oxygen capacity and hemoglobin values were obtained for the blood of eighteen species of mammals. These included man, seven species of common laboratory animals, six species of domestic livestock and four wild species. The laboratory animals were Strain A mice, Osborn-Mendel rats, hamsters, guinea pigs, rabbits, cats and dogs. The livestock were goats, sheep, swine, cows, mules and horses. The wild species were opossum, (*Didelphys virginiana*), cotton rat, (*Sigmodon hispidus*), deer mice (*Peromyscus leucopus*) and the eastern pipistrel (bat) (*Pipistrellus subflavus*).

The blood samples were drawn into syringes coated with heparin to prevent coagulation, and were kept in plastic containers at 3°C. The blood was used the same day it was drawn.

Hemoglobin determinations were made according to the method developed by Drabkin (1945, 1948 and 1949). This is a colorimetric procedure by which one compares the unknown sample with a series of carefully prepared standards of cyanmethemoglobin. According to the protocol given by Crosby, Munn and Furth (1954) duplicate samples were prepared by mixing 5.0 ml of Drabkin reagent and 0.02 ml of blood directly in the cuvettes. After standing for ten minutes, the optical density of each sample was read at 540 mμ.

Oxygen capacity determinations were obtained after equilibration of the samples with atmospheric air at 37.5°C. Blood gas analyses were made according to the manometric method of Holaday and Verosky (1956), using the Kopp-Natelson Microgasometer.

Results

The data obtained are presented in Table I. Each hemoglobin value quoted (column b) is the

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TABLE I

Species (a)	Phot. g. Hb/100 ml (b)	Gaso. O_2 cap. (c)	Calc. O_2 cap. (b × 1.36) (d)	Calc. O_2 cap. in % of gasometric (d/c) × 100	Gaso. O_2 cap./g. Hb (c/b)
Eastern pipistrel	15.00	19.85	20.40	102.9	1.323
White mouse	12.50	17.32	17.00	98.2	1.386
	12.10	15.38	16.46	107.0	1.308
Deer mouse	12.20	15.31	16.59	108.4	1.255
Cotton rat	9.70	14.53	13.19	90.8	1.498
	11.60	15.06	15.77	104.7	1.298
Hamster	14.10	19.16	19.18	100.1	1.359
	14.90	20.81	20.26	97.4	1.397
White rat	12.00	16.87	16.32	96.7	1.406
	13.20	17.06	17.95	105.2	1.292
Guinea pig	15.90	21.27	21.62	101.6	1.338
	12.50	16.16	17.00	105.2	1.293
Opossum	12.25	16.72	16.66	99.6	1.365
	12.30	15.58	16.73	107.4	1.267
Rabbit	11.75	16.25	15.98	98.3	1.383
	11.10	16.25	15.10	92.9	1.464
Cat	9.40	13.11	12.78	97.5	1.395
	10.20	14.30	13.87	97.0	1.402
Dog	16.79	22.93	22.83	99.6	1.366
	17.60	22.34	23.94	107.2	1.269
Goat	11.35	15.78	15.44	97.8	1.390
	10.00	13.84	13.60	98.3	1.384
Sheep	10.49	14.71	14.27	97.0	1.402
Man	14.87	20.28	20.22	99.7	1.371
	17.10	22.32	23.26	104.2	1.305
Swine	13.40	18.47	18.22	98.6	1.378
	12.70	17.39	17.27	99.3	1.369
Beef	16.00	21.72	21.76	100.2	1.358
	12.40	16.84	16.86	100.1	1.358
Mule	13.55	17.74	18.43	103.9	1.309
Horse	12.58	17.17	17.11	99.7	1.365
		17.50	17.60	m = 100.49	m = 1.36 sd. ± 0.06 se. ± 0.01

Phot. g. Hb/100 ml = photometrically determined hemoglobin.

Gaso. O_2 cap. = gasometrically determined oxygen capacity.

Calc. O_2 cap. = calculated oxygen capacity. Hb. × 1.36.

Calc. O_2 cap. in % of gasometric = calc. O_2 cap./gaso. O_2 cap. × 100.

Gaso. O_2 cap./g.Hb. = ml O_2 carried/g. phot. determined Hb.

average of duplicate analyses, and the gasometric oxygen capacities (column c) are averages of three or four determinations. The lowest values for hemoglobin were found for the blood of cotton rat, cat, sheep and goat, while the highest values were found for the blood of dog, man and beef. The gasometric oxygen capacity values ranged from 13.11 to 22.9 ml O_2/100 ml with a mean of 17.50.

An examination of columns (b) and (c) of Table I does not show a correlation between hemoglobin content or oxygen capacity values for the blood and body size. On the other hand, the data indicate a uniformity of the hemoglobins regarding their ability to carry oxygen. The calculated oxygen capacity data (column d) were arrived at by multiplying the values for hemoglobin (g./100 ml of blood) by the constant 1.36. The individual values for gasometric oxygen capacity (c) are in agreement with the calculated ones (d), and the mean values for the data obtained by the two methods are similar, being 17.50 by the direct method and 17.60 ml O_2/100 ml for the calculated values.

The calculated oxygen capacity (d) and the direct gasometric oxygen capacity (c) can be directly compared by expressing one as the percentage of the other as in column five (d/c × 100) Table I. The percentage difference between the data obtained by the two can be read directly from column five Table I. It can be seen that the calculated values are on the average 0.49 percent higher than the direct gasometric values.

The individual values for O_2/g. Hb obtained for each sample is indicated in the right-hand column (c/b) of Table I. They vary from 1.255 to 1.498, but the mean figure of 1.357 ± 0.055 is very near 1.36, which is the previously established value for the constant.

DISCUSSION

It is generally accepted that the hemes of all mammalian hemoglobins are identical, both in structure and physiological properties. Based strictly upon the data presented here, one cannot say, however, that all hemoglobins bind the same amount of oxygen per gram when completely saturated. It is possible only to state that they bind the same amount of oxygen per unit of light absorbed at 540 mμ by the cyanmethemoglobin form, when the oxygen content measurements are made by the method used here. This is emphasized since the size variation of the different

globin moieties of mammalian hemoglobins is not well known.

The value one obtains for the constant (ml O_2/100 ml) may vary slightly from the value 1.36, according to the method used for measuring the hemoglobin, or for the reverse procedure of determining the hemoglobin level from the oxygen capacity measurement, according to the method used in measuring the gasometric oxygen capacity. Barker (1957) used the value 1.35 for calculating oxygen capacity from the hemoglobin values of several species of mammals, while Nahas (1958) used the constant 1.34. With the method used to estimate hemoglobin in the present investigation, the value 1.36 is most satisfactory.

The use of hemoglobin values rather than gasometric determinations for oxygen capacity measurements offers an easier means of obtaining this value due to the simplicity of the photometric hemoglobin determinations compared to the gasometric measurements. In recent years, the use of photometric methods for determining the oxygen saturation of hemoglobin solutions or whole blood has become more prevalent. Any such data could easily be expressed as the gasometric equivalent after making appropriate hemoglobin analyses and applying the constant for converting the hemoglobin data to oxygen content values. In such determinations, however, one must be assured that the hemoglobin is in the physiologically active ferrohemoglobin form. This is particularly true of the method used here, since it measures both ferro- and ferrihemoglobin. The presence of non-active hemoglobin derivatives could perhaps account for the slightly higher mean O_2 capacity (0.49%) obtained from hemoglobin calculations as compared to gasometric measurements in the present study.

The hemoglobin level often increases as a compensatory adaptation in certain mammals living in environments of relative oxygen scarcity. An adaptation of this nature, where increased oxygen capacity accompanies diminished body size, should offer a means of delivering more oxygen to the tissues of small animals with high metabolic rates (Schmidt-Nielsen and Larimer, 1958). As was pointed out by Burke (1953) and as shown by the present investigator, such adaptations apparently have not occurred in those mammlas where an enhanced metabolic rate is a size-dependent function.

The constant 1.36 for converting hemoglobin values to oxygen capacities has only been used in comparative physiology in a few instances. Burke (1953) used this value to calculate the oxygen capacity of the blood of many different mammals. The choice of this constant was based on the original data of Bernhart and Skeegs (1943) who determined the value for human hemoglobin. It was assumed by Burke that the value is applicable for any mammalian hemoglobin. The present investigation indicates that this assumption is correct.

SUMMARY

Simultaneous hemoglobin and oxygen capacity estimates were made on the blood of eighteen species of mammals. A comparison was made between the oxygen capacity values which were arrived at by gasometric measurement and those estimated from the photometrically determined hemoglobin by multiplying by the constant 1.36. The mean calculated oxygen capacity was found to be 0.49% above the actual gasometric oxygen capacity. The ratio of gasometric oxygen capacity per gram of hemoglobin was found to be 1.36 ± 0.06 ml O_2/g. Hb.

The variations of the constant from the theoretical value of 1.36 ml O_2/g. Hb are probably due to the method of hemoglobin estimates or the contamination of the sample with hemoglobin derivatives which are not physiologically active in the transport of oxygen. It is therefore suggested that hemoglobin values and the constant 1.36 can be used to arrive at the oxygen capacity of the blood of any of the eighteen species used in the present investigation when the hemoglobin values have been estimated by the present technique.

Possible uses of the constant in spectrophotometric oxygen saturation studies and as a method of checking the validity of gasometric estimates have been suggested.

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